

## NARC8 PROGRAMMED CELL-DEATH-ASSOCIATED MOLECULES AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part application of copending U.S. Patent Application No. 09/692,785, filed October 20, 2000, and entitled "Nucleic Acid Molecules Derived from Rat Brain and Programmed Cell Death Models," which is based on U.S. Provisional Patent Application No. 60/161,188, filed 10/22/99, and entitled "Nucleic Acid Molecules Derived From Rat Brain and Programmed Cell Death Models." Both of these applications are hereby incorporated in their entirety by reference.

### FIELD OF THE INVENTION

The invention relates to novel human programmed cell death-associated sequences. Also provided are vectors, host cells, and recombinant methods for making and using the novel molecules.

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### BACKGROUND OF THE INVENTION

In multicellular organisms, homeostasis is maintained by balancing the rate of cell proliferation against the rate of cell death. Cell proliferation is influenced by numerous growth factors and the expression of proto-oncogenes, which typically encourage progression through the cell cycle. In contrast, numerous events, including the expression of tumor suppressor genes, can lead to an arrest of cellular proliferation.

In differentiated cells, programmed cell death (apoptosis) occurs when an internal suicide program is activated. This program can be initiated by a variety of external signals as well as by signals that are generated within the cell in response to, for example,

genetic damage. Dying cells are eliminated by phagocytes, without an inflammatory response.

Programmed cell death is a highly regulated process that involves transcription-dependent and -independent mechanisms (reviewed by Kaufman (1999) *Genes Dev.* 13:1211-1233; Dragunow and Preston (1995) *Brain Res. Rev.* 21:1-28; Raff *et al.* (1993) *Science* 286:2358-2361). A key molecular event in the process of programmed cell death is the activation of a signaling cascade mediated by the caspase family of serine proteases. Apoptotic signals include physiologic activators (growth factor deprivation, Fas activation, TGF- $\beta$ , etc.), damage-related inducers (heat shock, viral infection, bacterial toxins, oncogenes, oxidants, free radicals, etc.) therapy-associated agents (chemotherapeutic drugs, ionizing radiation, etc.) and toxins (including ethanol and  $\beta$ -amyloid peptide). These signals result in the conversion of the precursors of the caspases into proteolytically active enzymes, resulting in the activation of late effectors of morphological and physiological aspects of programmed cell death, including DNA fragmentation and cytoplasmic condensation. In addition, regulation of programmed cell death may be integrated with regulation of energy, redox- and ion homeostasis in the mitochondria (reviewed by Kroemer (1998) *Cell Death Differ.* 5:547), and/or cell-cycle control in the nucleus and cytoplasm (reviewed by Choisy-Rossi *et al.* (1998) *Cell Death Differ.* 5:129-131; Dang (1999) *Molec. Cell. Biol.* 19:1-11; Kasten *et al.* *Cell Death Differ.* 5:132-140). 1998)).

Apoptotic cells undergo an orchestrated cascade of events including the activation of endogenous proteases, loss of mitochondrial function, and structural changes, such as disruption of the cytoskeleton, cell shrinkage, membrane blebbing, and nuclear condensation due to degradation of DNA. The various signals that trigger programmed cell death may bring about these events by converging on a common cell death pathway that is regulated by the expression of genes that are highly conserved.

Programmed cell death is a normal physiological activity required for proper growth and differentiation in all vertebrates. Defects in apoptotic programs result in disorders including, but not limited to, neurodegenerative disorders, cancer, viral infections, AIDS (acquired immunodeficiency syndrome), heart disease and autoimmune diseases (Thompson *et al.* (1995) *Science* 267:1456).

In neurons, programmed cell death is an essential component of development , and has been associated with many forms of neurodegeneration (reviewed by Hetts (1998) *JAMA* 279:300-307; Pettmann *et al.* (1998) *Neuron* 20:633-647; Jacobson *et al.* (1997) *Cell* 88:347-354). In vertebrate species, neuronal programmed cell death  
5 mechanisms have been associated with a variety of developmental roles, including the removal of neuronal precursors which fail to establish appropriate synaptic connections (Oppenheim *et al.* (1991) *Annual Rev. Neuroscience* 14:453-501), the quantitative matching of pre- and post-synaptic population sizes (Herrup *et al.* (1987) *J. Neurosci.* 7:829-836), and sculpting of neuronal circuits, both during development and in the adult  
10 (Bottjer *et al.* (1992) *J. Neurobiol.* 23:1172-1191).

Inappropriate programmed cell death has been suggested to be involved in neuronal loss in various neurodegenerative diseases such as Alzheimer's disease (Loo *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:7951-7955), Huntington's disease (Portera-Cailliau *et al.* (1995) *J. Neurosci.* 15:3775-3787), amyotrophic lateral sclerosis (Rabizadeh *et al.*  
15 (1995) *Proc. Natl. Acad. Sci.* 92:3024-3028), and spinal muscular atrophy (Roy *et al.* (1995) *Cell* 80:167-178).

In addition, improper expression of genes involved in programmed cell death has been implicated in carcinogenesis. Several families of oncogenes have been shown to play a role in programmed cell death. One example is the Bcl-2 family of proteins, which  
20 plays a pivotal role in the committing step of programmed cell death.

Accordingly, genes involved in programmed cell death are important targets for therapeutic intervention. It is important, therefore, to identify novel genes involved in programmed cell death or to discover whether known genes function in this process.

## 25 SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery of novel human programmed cell death-associated proteins, referred to herein as "NARC8A" and "NARC8B" (or collectively as "NARC8"). The nucleotide sequence of a cDNA encoding NARC8A is shown in SEQ ID NO:1, and the amino acid sequence of a  
30 NARC8A polypeptide is shown in SEQ ID NO:2. In addition, the nucleotide sequence of the NARC8A coding region is depicted in SEQ ID NO:3. The nucleotide sequence of a

cDNA encoding NARC8B is shown in SEQ ID NO:4, and the amino acid sequence of a NARC8B polypeptide is shown in SEQ ID NO:5. In addition, the nucleotide sequence of the NARC8B coding region is depicted in SEQ ID NO:6.

Accordingly, in one aspect the invention features a nucleic acid molecule which  
5 encodes a NARC8 protein or polypeptide, e.g., a biologically active portion of the NARC8 (i.e. NARC8A or NARC8B) protein. In a preferred embodiment, the isolated nucleic acid molecule encodes a polypeptide having the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. In other embodiments, the invention provides an isolated NARC8 nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO:1,  
10 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_. In still other embodiments, the invention provides nucleic acid molecules that are substantially identical (e.g., naturally occurring allelic variants) to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA  
15 insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_ and \_\_\_\_\_. In other embodiments, the invention provides a nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC as Accession  
20 Numbers \_\_\_\_\_ or \_\_\_\_\_ wherein the nucleic acid encodes a full length NARC8 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include a NARC8 nucleic acid molecule described herein. In certain embodiments, the nucleic acid molecules of the invention are operatively linked to native or heterologous  
25 regulatory sequences. Also included, are vectors and host cells containing the NARC8 nucleic acid molecules of the invention e.g., vectors and host cells suitable for producing NARC8 nucleic acid molecules and polypeptides.

In another related aspect, the invention provides nucleic acid fragments suitable as primers or hybridization probes for the detection of NARC8-encoding nucleic acids.

30 In still another related aspect, isolated nucleic acid molecules that are antisense to a NARC8 encoding nucleic acid molecule are provided.

In another aspect, the invention features NARC8 polypeptides, and biologically active or antigenic fragments thereof that are useful, e.g., as reagents or targets in assays applicable to treatment and diagnosis of NARC8-mediated or -related disorders. In another embodiment, the invention provides NARC8 polypeptides having a NARC8 activity. Preferred polypeptides are NARC8 proteins having a NARC8 activity, e.g., a NARC8 activity as described herein and preferably having a nuclear receptor binding factor/mitochondrial release factor domain.

In other embodiments, the invention provides NARC8 polypeptides, e.g., a NARC8 polypeptide having the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5; the amino acid sequence encoded by the cDNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_ or \_\_\_\_; an amino acid sequence that is substantially identical to the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5; or an amino acid sequence encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3 SEQ ID NO:4, SEQ ID NO:6, or the sequence of the DNA insert of the plasmid deposited with ATCC Accession Number \_\_\_\_ or \_\_\_\_, wherein the nucleic acid encodes a full length NARC8 protein or an active fragment thereof.

In a related aspect, the invention further provides nucleic acid constructs which include a NARC8 nucleic acid molecule described herein.

In a related aspect, the invention provides NARC8 polypeptides or fragments operatively linked to non-NARC8 polypeptides to form fusion proteins.

In another aspect, the invention features antibodies and antigen-binding fragments thereof, that react with, or more preferably specifically bind NARC8 polypeptides.

In another aspect, the invention provides methods of screening for compounds that modulate the expression or activity of the NARC8 polypeptides or nucleic acids.

In still another aspect, the invention provides a process for modulating NARC8 polypeptide or nucleic acid expression or activity, e.g. using the screened compounds. In certain embodiments, the methods involve treatment of conditions related to aberrant activity or expression of the NARC8 polypeptides or nucleic acids, such as conditions involving aberrant or deficient cellular proliferation or differentiation.

The invention also provides assays for determining the activity of or the presence or absence of NARC8 polypeptides or nucleic acid molecules in a biological sample, including for disease diagnosis.

In further aspect the invention provides assays for determining the presence or absence of a genetic alteration in a NARC8 polypeptide or nucleic acid molecule, including for disease diagnosis.

### BRIEF DESCRIPTION OF THE DRAWINGS

*Figure 1* depicts a hydropathy plot of human NARC8A. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:2) of human NARC8A are indicated. Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

*Figure 2* depicts a hydropathy plot of human NARC8B. Relative hydrophobic residues are shown above the dashed horizontal line, and relative hydrophilic residues are below the dashed horizontal line. The cysteine residues (cys) and N glycosylation site (Ngly) are indicated by short vertical lines just below the hydropathy trace. The numbers corresponding to the amino acid sequence (shown in SEQ ID NO:5) of human NARC8B are indicated. Polypeptides of the invention include fragments which include: all or a part of a hydrophobic sequence (a sequence above the dashed line); or all or part of a hydrophilic fragment (a sequence below the dashed line). Other fragments include a cysteine residue or as N-glycosylation site.

*Figure 3* depicts an alignment of the zinc-binding dehydrogenase domain of human NARC8A with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence (SEQ ID NO:7), while the lower amino acid sequence corresponds to amino acids 1 to 297 of SEQ ID NO:2.

Figure 4 depicts an alignment of the zinc-binding dehydrogenase domain of human NARC8A with a consensus amino acid sequence derived from a hidden Markov model. The upper sequence is the consensus amino acid sequence (SEQ ID NO:7), while the lower amino acid sequence corresponds to amino acids 57 to 373 of SEQ ID NO:5.

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Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

#### DETAILED DESCRIPTION OF THE INVENTION

10 The present invention now will be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these  
15 fully convey the scope of the invention to those skilled in the art. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the invention will come to mind to one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be  
20 understood that the invention is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

#### 25 Human NARC8

The present invention is based, in part, on the discovery of novel human programmed cell death-associated proteins, referred to herein as "NARC8A" and "NARC8B" (or collectively as "NARC8"). While the present invention is not held to any particular genomic source for the NARC8A and NARC8B transcripts, these nucleotide  
30 sequences appear to correspond to alternate splice variants derived from the same gene.

The human NARC8A sequence (SEQ ID NO:1), which is approximately 1507 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 894 nucleotides (nucleotides 368-1261 of SEQ ID NO:1; SEQ ID NO:3). The coding sequence encodes a 297 amino acid protein (SEQ ID NO:2).

5 The human NARC8B sequence (SEQ ID NO:4), which is approximately 1380 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1122 nucleotides (nucleotides 13-1134 of SEQ ID NO:4; SEQ ID NO:6). The coding sequence encodes a 373 amino acid protein (SEQ ID NO:5).

Human NARC8 contains the following regions or other structural features: a  
10 predicted zinc-binding dehydrogenase domain (PFAM Accession PF00107; SEQ ID NO:7) located at about amino acid residues 1-297 of NARC8A (SEQ ID NO:2) and amino acids 57-373 of NARC8B (SEQ ID NO:5).

The NARC8 protein also includes the following domains (as determined by ProDom analysis): nuclear receptor binding factor/mitochondrial release factor (amino  
15 acids 2-32 and 33-267 of NARC8A (SEQ ID NO:2); amino acids 41-108 and 109-343 of NARC8B (SEQ ID NO:5)) and alcohol dehydrogenase/zinc dehydrogenase (amino acids 3-131 of NARC8A (SEQ ID NO:2); amino acids 41-207 of NARC 8B (SEQ ID NO:5)).

For general information regarding PFAM identifiers, PS prefix and PF prefix domain identification numbers, refer to Sonnhammer *et al.* (1997) *Protein* 28:405-420  
20 and <http://www.psc.edu/general/software/packages/pfam/pfam.html>.

Plasmids containing the nucleotide sequence encoding human NARC8A and human NARC8B were deposited with American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on \_\_\_\_\_ and assigned Accession Number \_\_\_\_\_. This deposit will be maintained under the terms of the Budapest Treaty  
25 on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

The NARC8 molecules of the invention are associated with programmed cell death. For example, overexpression of the rat homolog of human NARC8A in cerebellar  
30 granule neurons resulted in an approximately 6 fold increase in the number of apoptotic cells (see Example 2).



The activation of programmed cell death is manifested by changes including membrane blebbing, DNA fragmentation, cytoplasmic and nuclear degradation, chromatin aggregation, formation of apoptotic bodies, and cell death. Failure to appropriately activate programmed cell death can be manifested by changes including increased cell proliferation. Proteins and/or antibodies of the invention are also useful in modulating the apoptotic process.

The disclosed invention relates to methods and compositions for the modulation, diagnosis, and treatment of disorders associated with the inhibition of apoptosis, increased apoptosis, or with disruptions in the cell cycle.

Disorders associated with an inappropriately low rate of programmed cell death may prolong survival of abnormal cells (Thompson (1995) *Science* 267:1456-1462). These accumulated cells can give rise to cancers, including follicular lymphomas, carcinomas with *p53* mutations, or hormone-dependent tumors, such as breast, prostate, or ovarian cancers. Some viruses, including herepesviruses, poxviruses, and adenoviruses, have been shown to inhibit programmed cell death. Disabling this cellular defense mechanism allows the virus to propagate.

Primary apoptotic deficiency disorders include graft rejection. Accordingly, the invention is relevant to the identification of genes useful in inhibiting graft rejection. Further, it has been suggested that all autoimmune disorders can be viewed as primary deficiencies of apoptosis (Hetts (1998) *JAMA* 279:300-307). For example, autoimmune disorders, including systemic lupus erythematosus and immune-mediated glomerulonephritis, can arise if autoreactive lymphocytes are not removed following an immune response. Accordingly, the invention is relevant for screening for gene expression and transcriptional profiling in any autoimmune disorder and for screening for agents that affect the expression or transcriptional profile of these genes. Additional examples of such autoimmune disorders include autoimmune diabetes, local self reactive disorder (including Hashimoto thyroiditis), lymphoproliferation, and Canale-Smith syndrome.

Primary apoptosis excesses are associated with heart disease including idiopathic dilated cardiomyopathy, ischemic cardiomyopathy, and valvular heart disease. Evidence

has also been shown of apoptosis in heart failure resulting from arrhythmogenic right ventricular dysplasia. For all these disorders, see Hetts, above.

5 A wide variety of neurological diseases are characterized by the gradual loss of specific sets of neurons. Such disorders include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) retinitis pigmentosa, spinal muscular atrophy, and various forms of cerebellar degeneration. The cell loss in these diseases does not induce an inflammatory response, and apoptosis appears to be the mechanism of cell death.

10 In addition, a number of hematologic diseases are associated with a decreased production of blood cells. These disorders include anemia associated with chronic disease, aplastic anemia, chronic neutropenia, and the myelodysplastic syndromes. Disorders of blood cell production, such as myelodysplastic syndrome and some forms of aplastic anemia, are associated with increased apoptotic cell death within the bone marrow. These disorders could result from the activation of genes that promote apoptosis, acquired deficiencies in stromal cells or hematopoietic survival factors, or the  
15 direct effects of toxins and mediators of immune responses.

Two common disorders associated with cell death are myocardial infarctions and stroke. In both disorders, cells within the central area of ischemia, which is produced in the event of acute loss of blood flow, appear to die rapidly as a result of necrosis. However, outside the central ischemic zone, cells die over a more protracted time period  
20 and morphologically appear to die by apoptosis.

As used herein, the term "programmed cell death-associated protein" or "programmed cell death-associated polypeptide" refers to a protein or polypeptide which is capable of modulating (*i.e.* increasing or decreasing) the level of programmed cell death.

25 As the NARC8 polypeptides of the invention may modulate NARC8-mediated activities, they may be useful as of for developing novel diagnostic and therapeutic agents for NARC8-mediated or related disorders, as described below.

As used herein, a "NARC8 activity", "biological activity of NARC8" or "functional activity of NARC8", refers to an activity exerted by a NARC8 protein,  
30 polypeptide or nucleic acid molecule on e.g., a NARC8-responsive cell or on a NARC8 substrate, as determined *in vivo* or *in vitro*. In one embodiment, a NARC8 activity is a

direct activity, such as an association with a NARC8 target molecule. A "target molecule" or "binding partner" is a molecule with which a NARC8 protein binds or interacts in nature. A NARC8 activity can also be an indirect activity, e.g., a cellular signaling activity mediated by interaction of the NARC8 protein with a NARC8 target molecule or binding partner. For example, the NARC8 proteins of the present invention can have one or more of the following activities: 1) the modulation of cell proliferation, 2) the modulation of programmed cell death, 3) the induction of DNA fragmentation, 4) the modulation of cytoplasmic and nuclear degradation, 5) the induction of chromatin aggregation, and 6) the formation of apoptotic bodies

Accordingly, NARC8 protein may be mediate various disorders, including disorders associated with cell proliferation and aberrance in programmed cell death.

Examples of cellular proliferative and/or differentiative disorders include cancer, e.g., carcinoma, sarcoma, metastatic disorders or hematopoietic neoplastic disorders, e.g., leukemias. A metastatic tumor can arise from a multitude of primary tumor types, including but not limited to those of prostate, colon, lung, breast and liver origin.

As used herein, the terms "cancer", "hyperproliferative" and "neoplastic" refer to cells having the capacity for autonomous growth, i.e., an abnormal state or condition characterized by rapidly proliferating cell growth. Hyperproliferative and neoplastic disease states may be categorized as pathologic, i.e., characterizing or constituting a disease state, or may be categorized as non-pathologic, i.e., a deviation from normal but not associated with a disease state. The term is meant to include all types of cancerous growths or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness.

"Pathologic hyperproliferative" cells occur in disease states characterized by malignant tumor growth. Examples of non-pathologic hyperproliferative cells include proliferation of cells associated with wound repair.

The terms "cancer" or "neoplasms" include malignancies of the various organ systems, such as affecting lung, breast, thyroid, lymphoid, gastrointestinal, and genito-urinary tract, as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus.

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The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues including respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, breast carcinomas, prostatic carcinomas, endocrine system carcinomas, and melanomas. Exemplary  
5 carcinomas include those forming from tissue of the cervix, lung, prostate, breast, head and neck, colon and ovary. The term also includes carcinosarcomas, e.g., which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures.

10 The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation.

The NARC8 nucleic acid and protein of the invention can be used to treat and/or diagnose a variety of proliferative disorders. E.g., such disorders include hematopoietic neoplastic disorders. As used herein, the term "hematopoietic neoplastic disorders"  
15 includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin, e.g., arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Preferably, the diseases arise from poorly differentiated acute leukemias, e.g., erythroblastic leukemia and acute megakaryoblastic leukemia. Additional exemplary myeloid disorders include, but are not limited to, acute promyeloid leukemia (APML),  
20 acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus, L. (1991) *Crit. Rev. in Oncol./Hematol.* 11:267-97); lymphoid malignancies include, but are not limited to acute lymphoblastic leukemia (ALL) which includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL) and Waldenstrom's  
25 macroglobulinemia (WM). Additional forms of malignant lymphomas include, but are not limited to non-Hodgkin lymphoma and variants thereof, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and Reed-Sternberg disease.

The rat homolog of human NARC 8A was isolated from a rat brain cDNA library,  
30 thus the NARC8 molecules of the invention may be used to treat disorders of the brain. Such disorders include, but are not limited to, disorders involving neurons, and disorders

involving glia, such as astrocytes, oligodendrocytes, ependymal cells, and microglia; cerebral edema, raised intracranial pressure and herniation, and hydrocephalus; malformations and developmental diseases, such as neural tube defects, forebrain anomalies, posterior fossa anomalies, and syringomyelia and hydromyelia; perinatal brain injury; cerebrovascular diseases, such as those related to hypoxia, ischemia, and infarction, including hypotension, hypoperfusion, and low-flow states--global cerebral ischemia and focal cerebral ischemia--infarction from obstruction of local blood supply, intracranial hemorrhage, including intracerebral (intraparenchymal) hemorrhage, subarachnoid hemorrhage and ruptured berry aneurysms, and vascular malformations, hypertensive cerebrovascular disease, including lacunar infarcts, slit hemorrhages, and hypertensive encephalopathy; infections, such as acute meningitis, including acute pyogenic (bacterial) meningitis and acute aseptic (viral) meningitis, acute focal suppurative infections, including brain abscess, subdural empyema, and extradural abscess, chronic bacterial meningoencephalitis, including tuberculosis and mycobacterioses, neurosyphilis, and neuroborreliosis (Lyme disease), viral meningoencephalitis, including arthropod-borne (Arbo) viral encephalitis, *Herpes simplex* virus Type 1, *Herpes simplex* virus Type 2, *Varicella-zoster* virus (*Herpes zoster*), cytomegalovirus, poliomyelitis, rabies, and human immunodeficiency virus 1, including HIV-1 meningoencephalitis (subacute encephalitis), vacuolar myelopathy, AIDS-associated myopathy, peripheral neuropathy, and AIDS in children, progressive multifocal leukoencephalopathy, subacute sclerosing panencephalitis, fungal meningoencephalitis, other infectious diseases of the nervous system; transmissible spongiform encephalopathies (prion diseases); demyelinating diseases, including multiple sclerosis, multiple sclerosis variants, acute disseminated encephalomyelitis and acute necrotizing hemorrhagic encephalomyelitis, and other diseases with demyelination; degenerative diseases, such as degenerative diseases affecting the cerebral cortex, including Alzheimer disease and Pick disease, degenerative diseases of basal ganglia and brain stem, including Parkinsonism, idiopathic Parkinson disease (paralysis agitans), progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, including striatonigral degeneration, Shy-Drager syndrome, and olivopontocerebellar atrophy, and Huntington disease; spinocerebellar degenerations, including

spinocerebellar ataxias, including Friedreich ataxia, and ataxia-telangiectasia, degenerative diseases affecting motor neurons, including amyotrophic lateral sclerosis (motor neuron disease), bulbospinal atrophy (Kennedy syndrome), and spinal muscular atrophy; inborn errors of metabolism, such as leukodystrophies, including Krabbe

5 disease, metachromatic leukodystrophy, adrenoleukodystrophy, Pelizaeus-Merzbacher disease, and Canavan disease, mitochondrial encephalomyopathies, including Leigh disease and other mitochondrial encephalomyopathies; toxic and acquired metabolic diseases, including vitamin deficiencies such as thiamine (vitamin B<sub>1</sub>) deficiency and vitamin B<sub>12</sub> deficiency, neurologic sequelae of metabolic disturbances, including

10 hypoglycemia, hyperglycemia, and hepatic encephatopathy, toxic disorders, including carbon monoxide, methanol, ethanol, and radiation, including combined methotrexate and radiation-induced injury; tumors, such as gliomas, including astrocytoma, including fibrillary (diffuse) astrocytoma and glioblastoma multiforme, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and brain stem glioma, oligodendroglioma, and

15 ependymoma and related paraventricular mass lesions, neuronal tumors, poorly differentiated neoplasms, including medulloblastoma, other parenchymal tumors, including primary brain lymphoma, germ cell tumors, and pineal parenchymal tumors, meningiomas, metastatic tumors, paraneoplastic syndromes, peripheral nerve sheath tumors, including schwannoma, neurofibroma, and malignant peripheral nerve sheath

20 tumor (malignant schwannoma), and neurocutaneous syndromes (phakomatoses), including neurofibromatosis, including Type 1 neurofibromatosis (NF1) and TYPE 2 neurofibromatosis (NF2), tuberous sclerosis, and Von Hippel-Lindau disease.

The NARC8A and NARC8B proteins, fragments thereof, and derivatives and other variants of the sequence in SEQ ID NO:2 and SEQ ID NO:5 are collectively

25 referred to as "polypeptides or proteins of the invention" or "NARC8 polypeptides or proteins". Nucleic acid molecules encoding such polypeptides or proteins are collectively referred to as "nucleic acids of the invention" or "NARC8 nucleic acids." NARC8 molecules refer to NARC8 nucleic acids, polypeptides, and antibodies.

As used herein, the term "nucleic acid molecule" includes DNA molecules (e.g., a

30 cDNA or genomic DNA) and RNA molecules (e.g., an mRNA) and analogs of the DNA

or RNA generated, e.g., by the use of nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

The term "isolated or purified nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regards to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and/or 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of 5' and/or 3' nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized.

As used herein, the term "hybridizes under stringent conditions" describes conditions for hybridization and washing. Stringent conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology* John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. A preferred, example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50°C. Another example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 55°C. A further example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C. Preferably, stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more

washes in 0.2X SSC, 0.1% SDS at 65°C. Particularly preferred stringency conditions (and the conditions that should be used if the practitioner is uncertain about what conditions should be applied to determine if a molecule is within a hybridization limitation of the invention) are 0.5M Sodium Phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6 corresponds to a naturally-occurring nucleic acid molecule.

As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a NARC8 protein, preferably a mammalian NARC8 protein, and can further include non-coding regulatory sequences, and introns.

An "isolated" or "purified" polypeptide or protein is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. In one embodiment, the language "substantially free" means preparation of NARC8 protein or polypeptide having less than about 30%, 20%, 10% and more preferably 5% (by dry weight), of non-NARC8 protein (also referred to herein as a "contaminating protein"), or of chemical precursors or non-NARC8 chemicals. When the NARC8 protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation. The invention includes isolated or purified preparations of at least 0.01, 0.1, 1.0, and 10 milligrams in dry weight.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence encoding NARC8 (e.g., the sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_ or \_\_\_\_\_) without



abolishing or more preferably, without substantially altering a biological activity, whereas an "essential" amino acid residue results in such a change. For example, amino acid residues that are conserved among the polypeptides of the present invention are predicted to be particularly unamenable to alteration.

5 A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, 10 valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in a NARC8 protein is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be 15 introduced randomly along all or part of a NARC8 coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for NARC8 biological activity to identify mutants that retain activity. Following mutagenesis of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Numbers \_\_\_\_\_ or \_\_\_\_\_, 20 the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

As used herein, a "biologically active portion" of a NARC8 protein includes a fragment of a NARC8 protein which participates in an interaction between a NARC8 25 molecule and a non-NARC8 molecule. Biologically active portions of a NARC8 protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the NARC8 protein, e.g., the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5, which include less amino acids than the full length NARC8 proteins, and exhibit at least one activity of a NARC8 protein. Typically, 30 biologically active portions comprise a domain or motif with at least one activity of the NARC8 protein, e.g., programmed cell death-associated protein activity. A biologically

active portion of a NARC8 protein can be a polypeptide which is, for example, 10, 25, 50, 100, 200 or more amino acids in length. Biologically active portions of a NARC8 protein can be used as targets for developing agents which modulate a NARC8 mediated activity, e.g., programmed cell death-associated activity.

5           Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid  
10   sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 90%, 100% of the length of the reference sequence (e.g., when aligning a second  
15   sequence to the NARC8A amino acid sequence of SEQ ID NO:2, 89 amino acid residues, at least 120, preferably at least 148, more preferably at least 178, even more preferably at least 208, and even more preferably at least 238, 268, or 297 amino acid residues are aligned; and when aligning a second sequence to the NARC8B amino acid sequence of  
20   SEQ ID NO:5, 112 amino acid residues, at least 149, preferably at least 186, more preferably at least 224, even more preferably at least 261, and even more preferably at least 298, 336, or 373 amino acid residues are aligned. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the  
25   molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

30           The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred

embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (1970) *J. Mol. Biol.* 48:444-453 algorithm which has been incorporated into the GAP program in the GCG software package (available at <http://www.gcg.com>), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about what parameters should be applied to determine if a molecule is within a sequence identity or homology limitation of the invention) is using a Blossum 62 scoring matrix with a gap open penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The percent identity between two amino acid or nucleotide sequences can be determined using the algorithm of E. Meyers and W. Miller (1989) *CABIOS* 4:11-17 which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

The nucleic acid and protein sequences described herein can be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to NARC8 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to NARC8 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See <http://www.ncbi.nlm.nih.gov>.

"Misexpression or aberrant expression", as used herein, refers to a non-wild type pattern of gene expression, at the RNA or protein level. It includes: expression at non-wild type levels, i.e., over or under expression; a pattern of expression that differs from wild type in terms of the time or stage at which the gene is expressed, e.g., increased or decreased expression (as compared with wild type) at a predetermined developmental period or stage; a pattern of expression that differs from wild type in terms of decreased expression (as compared with wild type) in a predetermined cell type or tissue type; a pattern of expression that differs from wild type in terms of the splicing size, amino acid sequence, post-translational modification, or biological activity of the expressed polypeptide; a pattern of expression that differs from wild type in terms of the effect of an environmental stimulus or extracellular stimulus on expression of the gene, e.g., a pattern of increased or decreased expression (as compared with wild type) in the presence of an increase or decrease in the strength of the stimulus.

"Treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

A "therapeutic agent" includes, but is not limited to, small molecules, peptides, antibodies, ribozymes and antisense oligonucleotides.

"Subject", as used herein, can refer to a mammal, e.g., a human, or to an experimental or animal or disease model. The subject can also be a non-human animal, e.g., a horse, cow, goat, or other domestic animal.

A "purified preparation of cells", as used herein, refers to, in the case of plant or animal cells, an in vitro preparation of cells and not an entire intact plant or animal. In the case of cultured cells or microbial cells, it consists of a preparation of at least 10% and more preferably 50% of the subject cells.

Various aspects of the invention are described in further detail below.

### Isolated Nucleic Acid Molecules

In one aspect, the invention provides, an isolated or purified, nucleic acid molecule that encodes a NARC8 (i.e. NARC8A or NARC8B) polypeptide described herein, e.g., a full length NARC8 protein or a fragment thereof, e.g., a biologically active  
5 portion of NARC8 protein. Also included is a nucleic acid fragment suitable for use as a hybridization probe, which can be used, e.g., to identify nucleic acid molecule encoding a polypeptide of the invention, NARC8 mRNA, and fragments suitable for use as primers, e.g., PCR primers for the amplification or mutation of nucleic acid molecules.

In one embodiment, an isolated nucleic acid molecule of the invention includes  
10 the nucleotide sequence shown in SEQ ID NO:1, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human NARC8A protein (i.e., "the coding region", from nucleotides 368-1261 of SEQ ID NO:1), as well as 5' untranslated sequences  
15 (nucleotides 1-367 of SEQ ID NO:1). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., nucleotides 368-1261 of SEQ ID NO:1, corresponding to SEQ ID NO:3) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:2.

20 In another embodiment, an isolated nucleic acid molecule of the invention includes the nucleotide sequence shown in SEQ ID NO:4, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, or a portion of any of these nucleotide sequences. In one embodiment, the nucleic acid molecule includes sequences encoding the human NARC8B protein (i.e., "the coding  
25 region", from nucleotides 13-1134 of SEQ ID NO:4), as well as 5' untranslated sequences (nucleotides 1-12 of SEQ ID NO:4). Alternatively, the nucleic acid molecule can include only the coding region of SEQ ID NO:1 (e.g., nucleotides 13-1134 of SEQ ID NO:1, corresponding to SEQ ID NO:6) and, e.g., no flanking sequences which normally accompany the subject sequence. In another embodiment, the nucleic acid  
30 molecule encodes a sequence corresponding to the mature protein of SEQ ID NO:5.

In another embodiment, an isolated nucleic acid molecule of the invention includes a nucleic acid molecule which is a complement of the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, or a portion of any of these nucleotide sequences. In other  
5       embodiments, the nucleic acid molecule of the invention is sufficiently complementary to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_ such that it can hybridize to the nucleotide  
10       sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, thereby forming a stable duplex.

In one embodiment, an isolated nucleic acid molecule of the present invention includes a nucleotide sequence which is at least about 60%, 65%, 70%, 75%, 80%, 85%,  
15       90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_. In the case of an isolated nucleic acid molecule which is longer than or equivalent in length to the reference sequence, e.g., SEQ ID  
20       NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6, the comparison is made with the full length of the reference sequence. Where the isolated nucleic acid molecule is shorter than the reference sequence, e.g., shorter than SEQ ID NO:1, SEQ ID NO:3, or SEQ ID NO:4, the comparison is made to a segment of the reference sequence of the same length (excluding any loop required by the homology calculation).

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#### NARC8 Nucleic Acid Fragments

A nucleic acid molecule of the invention can include only a portion of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession  
30       Number \_\_\_\_\_ or \_\_\_\_\_. For example, such a nucleic acid molecule can include a fragment which can be used as a probe or primer or a fragment encoding a portion of a

NARC8 protein, e.g., an immunogenic or biologically active portion of a NARC8 protein. A fragment can comprise, for example, nucleotides 371-1169 of SEQ ID NO:1, or nucleotides 133-1041 of SEQ ID NO:4, which encode a nuclear receptor binding factor/mitochondrial release factor domain of human NARC8. The nucleotide sequence  
5 determined from the cloning of the NARC8 cDNA allows for the generation of probes and primers designed for use in identifying and/or cloning other NARC8 family members, or fragments thereof, as well as NARC8 homologues, or fragments thereof, from other species.

In another embodiment, a nucleic acid includes a nucleotide sequence that includes  
10 part, or all, of the coding region and extends into either (or both) the 5' or 3' noncoding region. Other embodiments include a fragment which includes a nucleotide sequence encoding an amino acid fragment described herein. Nucleic acid fragments can encode a specific domain or site described herein or fragments thereof, particularly fragments thereof which are at least 150 amino acids in length. Fragments also include nucleic acid sequences  
15 corresponding to specific amino acid sequences described above or fragments thereof. Nucleic acid fragments should not to be construed as encompassing those fragments that may have been disclosed prior to the invention.

A nucleic acid fragment can include a sequence corresponding to a domain, region, or functional site described herein. A nucleic acid fragment can also include one or more  
20 domain, region, or functional site described herein. Thus, for example, the nucleic acid fragment can include a zinc-binding dehydrogenase domain or a nuclear receptor binding factor/mitochondrial release factor domain. In a preferred embodiment the fragment is at least, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500 base pairs in length.

NARC8 probes and primers are provided. Typically a probe/primer is an isolated  
25 or purified oligonucleotide. The oligonucleotide typically includes a region of nucleotide sequence that hybridizes under stringent conditions to at least about 7, 12 or 15, preferably about 20 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 consecutive nucleotides of a sense or antisense sequence of SEQ ID NO:1, SEQ ID  
30 NO:3, SEQ ID NO:4, SEQ ID NO:7, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, or of a naturally

occurring allelic variant or mutant of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_.

5 In a preferred embodiment the nucleic acid is a probe which is at least 5 or 10, and less than 200, more preferably less than 100, or less than 50, base pairs in length. It should be identical, or differ by 1, or less than in 5 or 10 bases, from a sequence disclosed herein. If alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

10 A probe or primer can be derived from the sense or anti-sense strand of a nucleic acid which encodes a programmed cell death-associated protein.

In another embodiment, a set of primers is provided, e.g., primers suitable for use in a PCR, which can be used to amplify a selected region of a NARC8 sequence, e.g., a region described herein. The primers should be at least 5, 10, or 50 base pairs in length  
15 and less than 100, or less than 200, base pairs in length. The primers should be identical, or differs by one base from a sequence disclosed herein or from a naturally occurring variant. E.g., primers suitable for amplifying all or a portion of any of the following regions are provided: a nuclear receptor binding factor/mitochondrial release factor domain (e.g., about amino acid residues 2-267 of SEQ ID NO:2 or 41-343 of SEQ ID  
20 NO:5).

A nucleic acid fragment can encode an epitope bearing region of a polypeptide described herein.

A nucleic acid fragment encoding a "biologically active portion of a NARC8 polypeptide" can be prepared by isolating a portion of the nucleotide sequence of SEQ ID  
25 NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, which encodes a polypeptide having a NARC8 biological activity (e.g., the biological activities of the NARC8 proteins as described herein), expressing the encoded portion of the NARC8 protein (e.g., by recombinant expression *in vitro*) and assessing the activity  
30 of the encoded portion of the NARC8 protein. For example, a nucleic acid fragment encoding a biologically active portion of NARC8 includes a nuclear receptor binding



factor/mitochondrial release factor protein domain (e.g., about amino acid residues 2-267 of SEQ ID NO:2 or amino acid residues 41-343 of SEQ ID NO:5). A nucleic acid fragment encoding a biologically active portion of a NARC8 polypeptide, may comprise a nucleotide sequence which is greater than 300-1200 or more nucleotides in length.

5 In preferred embodiments, nucleic acids include a nucleotide sequence that is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, or 1500 nucleotides in length and hybridizes under stringent hybridization conditions to a nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:3, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_, as well a  
10 nucleotide sequence that is about 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, or 1380 nucleotides in length and hybridizes under stringent conditions to a nucleic acid molecule of SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_.

#### 15 NARC8 Nucleic Acid Variants

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_. Such differences can be due to degeneracy of the  
20 genetic code (and result in a nucleic acid which encodes the same NARC8 proteins as those encoded by the nucleotide sequence disclosed herein. In another embodiment, an isolated nucleic acid molecule of the invention has a nucleotide sequence encoding a protein having an amino acid sequence which differs, by at least 1, but less than 5, 10, 20, 50, or 100 amino acid residues that is shown in SEQ ID NO:2 or SEQ ID NO:5. If  
25 alignment is needed for this comparison the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Nucleic acids of the invention can be chosen for having codons, which are preferred, or non preferred, for a particular expression system. E.g., the nucleic acid can  
30 be one in which at least one codon, at preferably at least 10%, or 20% of the codons has

been altered such that the sequence is optimized for expression in *E. coli*, yeast, human, insect, or CHO cells.

5 Nucleic acid variants can be naturally occurring, such as allelic variants (same locus), homologs (different locus), and orthologs (different organism) or can be non-naturally occurring. Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. The variations can produce both conservative and non-conservative amino acid substitutions (as compared in the encoded  
10 product).

In a preferred embodiment, the nucleic acid differs from that of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, e.g., as follows:  
15 by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the in the subject nucleic acid. If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.

Orthologs, homologs, and allelic variants can be identified using methods known in the art. These variants comprise a nucleotide sequence encoding a polypeptide that is 50%,  
20 at least about 55%, typically at least about 70-75%, more typically at least about 80-85%, and most typically at least about 90-95% or more identical to the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:5, or a fragment of one of these sequences. Such nucleic acid molecules can readily be obtained as being able to hybridize under stringent conditions, to the nucleotide sequence shown in SEQ ID NO:3, SEQ ID NO:6, or a  
25 fragment of one of these sequences. Nucleic acid molecules corresponding to orthologs, homologs, and allelic variants of the NARC8 cDNAs of the invention can further be isolated by mapping to the same chromosome or locus as the NARC8 gene. Preferred variants include those that are correlated with programmed cell death-associated protein activity.

30 Allelic variants of NARC8, e.g., human NARC8, include both functional and non-functional proteins. Functional allelic variants are naturally occurring amino acid

sequence variants of the NARC8 protein within a population that maintain the ability to modulate programmed cell death or cell proliferation. Functional allelic variants will typically contain only conservative substitution of one or more amino acids of SEQ ID NO:2 or SEQ ID NO:5, or substitution, deletion or insertion of non-critical residues in non-critical regions of the protein. Non-functional allelic variants are naturally-occurring amino acid sequence variants of the NARC8, e.g., human NARC8, protein within a population that do not have the ability to modulate programmed cell death. Non-functional allelic variants will typically contain a non-conservative substitution, a deletion, or insertion, or premature truncation of the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:5, or a substitution, insertion, or deletion in critical residues or critical regions of the protein.

Moreover, nucleic acid molecules encoding other NARC8 family members and, thus, which have a nucleotide sequence which differs from the NARC8 sequences of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, or the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_ are intended to be within the scope of the invention.

#### Antisense Nucleic Acid Molecules, Ribozymes and Modified NARC8 Nucleic Acid Molecules

In another aspect, the invention features, an isolated nucleic acid molecule which is antisense to NARC8. An "antisense" nucleic acid can include a nucleotide sequence which is complementary to a "sense" nucleic acid encoding a protein, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. The antisense nucleic acid can be complementary to an entire NARC8 coding strand, or to only a portion thereof (e.g., the coding region of human NARC8A (SEQ ID NO:3) or NARC8B (SEQ ID NO:6)). In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding NARC8 (e.g., the 5' and 3' untranslated regions).

An antisense nucleic acid can be designed such that it is complementary to the entire coding region of NARC8 mRNA, but more preferably is an oligonucleotide which

is antisense to only a portion of the coding or noncoding region of NARC8 mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of NARC8 mRNA, e.g., between the -10 and +10 regions of the target gene nucleotide sequence of interest. An antisense oligonucleotide can be, for example, about 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, or more nucleotides in length.

An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. The antisense nucleic acid also can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject (e.g., by direct injection at a tissue site), or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a NARC8 protein to thereby inhibit expression of the protein, e.g., by inhibiting transcription and/or translation. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an  $\alpha$ -anomeric nucleic acid molecule. An  $\alpha$ -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett.* 215:327-330).

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. A ribozyme having specificity for a NARC8-encoding nucleic acid can include one or more sequences complementary to the nucleotide sequence of a NARC8 cDNA disclosed herein (i.e., SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:6), and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Pat. No. 5,093,246 or Haselhoff and Gerlach (1988) *Nature* 334:585-591). For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a NARC8-encoding mRNA. See, e.g., Cech *et al.* U.S. Patent No. 4,987,071; and Cech *et al.* U.S. Patent No. 5,116,742. Alternatively, NARC8 mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W. (1993) *Science* 261:1411-1418.

NARC8 gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the NARC8 (e.g., the NARC8 promoter and/or enhancers) to form triple helical structures that prevent transcription of the NARC8 gene in target cells. See generally, Helene, C. (1991) *Anticancer Drug Des.* 6(6):569-84; Helene, C. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher, L.J. (1992) *Bioassays* 14(12):807-15. The potential sequences that can be targeted for triple helix formation can be increased by creating a so-called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

The invention also provides detectably labeled oligonucleotide primer and probe molecules. Typically, such labels are chemiluminescent, fluorescent, radioactive, or colorimetric.

A NARC8 nucleic acid molecule can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acid molecules can be modified to generate peptide nucleic acids (see Hyrup B. *et al.* (1996) *Bioorganic & Medicinal Chemistry* 4 (1): 5-23). As used herein, the terms "peptide nucleic acid" or "PNA" refers to a nucleic acid mimic, e.g., a DNA mimic, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of a PNA can allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *et al.* *Proc. Natl. Acad. Sci.* 93:14670-675.

PNAs of NARC8 nucleic acid molecules can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, for example, inducing transcription or translation arrest or inhibiting replication. PNAs of NARC8 nucleic acid molecules can also be used in the analysis of single base pair mutations in a gene, (e.g., by PNA-directed PCR clamping); as 'artificial restriction enzymes' when used in combination with other enzymes, (e.g., S1 nucleases (Hyrup B. (1996) *supra*)); or as probes or primers for DNA sequencing or hybridization (Hyrup B. *et al.* (1996) *supra*; Perry-O'Keefe *supra*).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, e.g., Letsinger *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (See, e.g., Krol *et al.* (1988) *Bio-Techniques* 6:958-976) or

intercalating agents. (See, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, (e.g., a peptide, hybridization triggered cross-linking agent, transport agent, or hybridization-triggered cleavage agent).

The invention also includes molecular beacon oligonucleotide primer and probe molecules having at least one region which is complementary to a NARC8 nucleic acid of the invention, two complementary regions one having a fluorophore and one a quencher such that the molecular beacon is useful for quantitating the presence of the NARC8 nucleic acid of the invention in a sample. Molecular beacon nucleic acids are described, for example, in Lizardi *et al.* U.S. Patent No. 5,854,033; Nazarenko *et al.* U.S. Patent No. 5,866,336, and Livak *et al.* U.S. Patent 5,876,930.

#### Isolated NARC8 Polypeptides

In another aspect, the invention features, an isolated NARC8 protein, or fragment, e.g., a biologically active portion, for use as immunogens or antigens to raise or test (or more generally to bind) anti-NARC8 antibodies. NARC8 protein can be isolated from cells or tissue sources using standard protein purification techniques. NARC8 protein or fragments thereof can be produced by recombinant DNA techniques or synthesized chemically.

Polypeptides of the invention include those which arise as a result of the existence of multiple genes, alternative transcription events, alternative RNA splicing events, and alternative translational and postranslational events. The polypeptide can be expressed in systems, e.g., cultured cells, which result in substantially the same postranslational modifications present when expressed the polypeptide is expressed in a native cell, or in systems which result in the alteration or omission of postranslational modifications, e.g., glycosylation or cleavage, present when expressed in a native cell.

In a preferred embodiment, a NARC8 polypeptide has one or more of the following characteristics:

- (i) it modulates programmed cell death (apoptosis);
- (ii) it has a molecular weight, e.g., a deduced molecular weight, amino acid composition or other physical characteristic of the polypeptide of SEQ ID NO:2 or SEQ ID NO:5;

(iii) it has an overall sequence identity of at least 50%, preferably at least 60%, more preferably at least 70, 80, 90, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, with a polypeptide of SEQ ID NO:2 or SEQ ID NO:5;

5 (iv) it has a nuclear receptor binding factor/mitochondrial release factor domain which preferably has an overall sequence identity of about 70%, 80%, 90% or 95% with amino acid residues 2-267 of SEQ ID NO:2 or amino acids 41-343 of SEQ ID NO:5;

(v) it has at least 70%, preferably 80%, and most preferably 95% of the cysteines found amino acid sequence of the native protein.

10 In a preferred embodiment the NARC8 protein, or fragment thereof, differs from the corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5. In one embodiment it differs by at least one but by less than 15, 10 or 5 amino acid residues. In another it differs from the corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5 by at least one residue but less than 20%, 15%, 10% or 5% of the residues in it differ from the  
15 corresponding sequence in SEQ ID NO:2 or SEQ ID NO:5. (If this comparison requires alignment the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences.) The differences are, preferably, differences or changes at a non-essential residue or a conservative substitution. In a preferred embodiment the differences are not in the  
20 nuclear receptor binding factor/mitochondrial release factor domain. In another preferred embodiment one or more differences are in non-active site residues.

Other embodiments include a protein that contain one or more changes in amino acid sequence, e.g., a change in an amino acid residue which is not essential for activity. Such NARC8 proteins differ in amino acid sequence from SEQ ID NO:2 or SEQ ID  
25 NO:5, yet retain biological activity.

In one embodiment, a biologically active portion of a NARC8 protein includes an zinc-binding dehydrogenase domain. In another embodiment, a biologically active portion of a NARC8 protein includes a nuclear receptor binding factor/mitochondrial release factor domain. Moreover, other biologically active portions, in which other  
30 regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native NARC8 protein.



In a preferred embodiment, the NARC8 protein has an amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5. In other embodiments, the NARC8 protein is substantially identical to SEQ ID NO:2 or SEQ ID NO:5. In yet another embodiment, the NARC8 protein is substantially identical to SEQ ID NO:2 or SEQ ID NO:5 and retains the functional activity of the protein of SEQ ID NO:2 or SEQ ID NO:5, as described in detail above. Accordingly, in another embodiment, the NARC8 protein is a protein which includes an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more identical to SEQ ID NO:2 or SEQ ID NO:5.

#### NARC8 Chimeric or Fusion Proteins

In another aspect, the invention provides NARC8 chimeric or fusion proteins. As used herein, a NARC8 "chimeric protein" or "fusion protein" includes a NARC8 polypeptide linked to a non-NARC8 polypeptide. A "non-NARC8 polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to the NARC8 protein, e.g., a protein which is different from the NARC8 protein and which is derived from the same or a different organism. The NARC8 polypeptide of the fusion protein can correspond to all or a portion e.g., a fragment described herein of a NARC8 amino acid sequence. In a preferred embodiment, a NARC8 fusion protein includes at least one (or two) biologically active portion of a NARC8 protein. The non-NARC8 polypeptide can be fused to the N-terminus or C-terminus of the NARC8 polypeptide.

The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST-NARC8 fusion protein in which the NARC8 sequences are fused to the C-terminus of the GST sequences. Such fusion proteins can facilitate the purification of recombinant NARC8. Alternatively, the fusion protein can be a NARC8 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of NARC8 can be increased through use of a heterologous signal sequence.

Fusion proteins can include all or a part of a serum protein, e.g., an IgG constant region, or human serum albumin.

The NARC8 fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject *in vivo*. The NARC8 fusion proteins can be used to affect the bioavailability of a NARC8 substrate. NARC8 fusion proteins may be useful therapeutically for the treatment of disorders caused by, for  
5 example, (i) aberrant modification or mutation of a gene encoding a NARC8 protein; (ii) misregulation of the NARC8 gene; and (iii) aberrant post-translational modification of a NARC8 protein.

Moreover, the NARC8-fusion proteins of the invention can be used as immunogens to produce anti-NARC8 antibodies in a subject, to purify NARC8 ligands  
10 and in screening assays to identify molecules which inhibit the interaction of NARC8 with a NARC8 substrate.

Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A NARC8-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the NARC8  
15 protein.

#### Variants of NARC8 Proteins

In another aspect, the invention also features a variant of a NARC8 polypeptide, e.g., which functions as an agonist (mimetics) or as an antagonist. Variants of the  
20 NARC8 proteins can be generated by mutagenesis, e.g., discrete point mutation, the insertion or deletion of sequences or the truncation of a NARC8 protein. An agonist of the NARC8 proteins can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of a NARC8 protein. An antagonist of a NARC8 protein can inhibit one or more of the activities of the naturally occurring form  
25 of the NARC8 protein by, for example, competitively modulating a NARC8-mediated activity of a NARC8 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Preferably, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally  
30 occurring form of the NARC8 protein.

5 Variants of a NARC8 protein can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of a NARC8 protein for agonist or antagonist activity.

Libraries of fragments e.g., N terminal, C terminal, or internal fragments, of a NARC8 protein coding sequence can be used to generate a variegated population of fragments for screening and subsequent selection of variants of a NARC8 protein.

6 Variants in which a cysteine residues is added or deleted or in which a residue which is glycosylated is added or deleted are particularly preferred.

7 Methods for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Recursive ensemble mutagenesis (REM), a new technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify NARC8 variants (Arkin and Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave *et al.* (1993) *Protein Engineering* 6(3):327-331).

Cell based assays can be exploited to analyze a variegated NARC8 library. For example, a library of expression vectors can be transfected into a cell line, e.g., a cell line, which ordinarily responds to NARC8 in a substrate-dependent manner. The transfected cells are then contacted with NARC8 and the effect of the expression of the mutant on signaling by the NARC8 substrate can be detected, e.g., by measuring programmed cell death-associated protein activity. Plasmid DNA can then be recovered from the cells which score for inhibition, or alternatively, potentiation of signaling by the NARC8 substrate, and the individual clones further characterized.

In another aspect, the invention features a method of making a NARC8 polypeptide, e.g., a peptide having a non-wild type activity, e.g., an antagonist, agonist, or super agonist of a naturally occurring NARC8 polypeptide, e.g., a naturally occurring NARC8 polypeptide. The method includes: altering the sequence of a NARC8 polypeptide, e.g., altering the sequence, e.g., by substitution or deletion of one or more residues of a non-conserved region, a domain or residue disclosed herein, and testing the altered polypeptide for the desired activity.

In another aspect, the invention features a method of making a fragment or analog of a NARC8 polypeptide a biological activity of a naturally occurring NARC8 polypeptide. The method includes: altering the sequence, e.g., by substitution or deletion of one or more residues, of a NARC8 polypeptide, e.g., altering the sequence of a non-conserved region, or a domain or residue described herein, and testing the altered polypeptide for the desired activity.

### Anti-NARC8 Antibodies

In another aspect, the invention provides an anti-NARC8 antibody. The term "antibody" as used herein refers to an immunoglobulin molecule or immunologically active portion thereof, i.e., an antigen-binding portion. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and F(ab')<sub>2</sub> fragments which can be generated by treating the antibody with an enzyme such as pepsin.

The antibody can be a polyclonal, monoclonal, recombinant, e.g., a chimeric or humanized, fully human, non-human, e.g., murine, or single chain antibody. In a preferred embodiment it has effector function and can fix complement. The antibody can be coupled to a toxin or imaging agent.

A full-length NARC8 protein or, antigenic peptide fragment of NARC8 can be used as an immunogen or can be used to identify anti-NARC8 antibodies made with other immunogens, e.g., cells, membrane preparations, and the like. The antigenic peptide of NARC8 should include at least 8 amino acid residues of the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NO:5 and encompasses an epitope of NARC8. Preferably, the antigenic peptide includes at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues.

Fragments of NARC8 which include, e.g., residues 230-250 of SEQ ID NO:2 or residues 310-330 of SEQ ID NO:5 can be used to make, e.g., used as immunogens, or used to characterize the specificity of an antibody or antibodies against what are believed to be hydrophilic regions of the NARC8 protein. Similarly, a fragment of NARC8 which includes, e.g., residues 110-130 of SEQ ID NO:2 or residues 190-210 or SEQ ID NO:5 can be used to make an antibody against what is believed to be a hydrophobic region of

the NARC8 protein; a fragment of NARC8 which includes residues 2-267 of SEQ ID NO:2 or 41-343 of SEQ ID NO:5 can be used to make an antibody against the nuclear receptor binding factor/mitochondrial release factor region of the NARC8 protein.

Antibodies reactive with, or specific for, any of these regions, or other regions or domains described herein are provided.

In a preferred embodiment the antibody fails to bind an Fc receptor, e.g. it is a type which does not support Fc receptor binding or has been modified, e.g., by deletion or other mutation, such that it does not have a functional Fc receptor binding region.

Preferred epitopes encompassed by the antigenic peptide are regions of NARC8 are located on the surface of the protein, e.g., hydrophilic regions, as well as regions with high antigenicity. For example, an Emini surface probability analysis of the human NARC8 protein sequence can be used to indicate the regions that have a particularly high probability of being localized to the surface of the NARC8 protein and are thus likely to constitute surface residues useful for targeting antibody production.

In a preferred embodiment the antibody binds an epitope on any domain or region on NARC8 proteins described herein.

Chimeric, humanized, but most preferably, completely human antibodies are desirable for applications which include repeated administration, e.g., therapeutic treatment (and some diagnostic applications) of human patients.

The anti-NARC8 antibody can be a single chain antibody. A single-chain antibody (scFV) may be engineered (see, for example, Colcher, D. *et al.* (1999, Jun 30) *Ann. NY Acad. Sci.* 880:263-80; and Reiter, Y. (1996 Feb) *Clin. Cancer Res.* 2(2):245-52). The single chain antibody can be dimerized or multimerized to generate multivalent antibodies having specificities for different epitopes of the same target NARC8 protein.

An anti-NARC8 antibody (e.g., monoclonal antibody) can be used to isolate NARC8 by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, an anti-NARC8 antibody can be used to detect NARC8 protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the protein. Anti-NARC8 antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can

be facilitated by coupling (i.e., physically linking) the antibody to a detectable substance (i.e., antibody labeling). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

#### Recombinant Expression Vectors, Host Cells and Genetically Engineered Cells

In another aspect, the invention includes, vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide described herein. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked and can include a plasmid, cosmid or viral vector. The vector can be capable of autonomous replication or it can integrate into a host DNA. Viral vectors include, e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses.

A vector can include a NARC8 nucleic acid in a form suitable for expression of the nucleic acid in a host cell. Preferably the recombinant expression vector includes one or more regulatory sequences operatively linked to the nucleic acid sequence to be expressed. The term "regulatory sequence" includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence, as well as tissue-specific regulatory and/or inducible sequences. The design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or polypeptides,

including fusion proteins or polypeptides, encoded by nucleic acids as described herein (e.g., NARC8 proteins, mutant forms of NARC8 proteins, fusion proteins, and the like).

The recombinant expression vectors of the invention can be designed for expression of NARC8 proteins in prokaryotic or eukaryotic cells. For example, polypeptides of the invention can be expressed in *E. coli*, insect cells (e.g., using baculovirus expression vectors), yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S. (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Purified fusion proteins can be used in NARC8 activity assays, (e.g., direct assays or competitive assays described in detail below), or to generate antibodies specific for NARC8 proteins. In a preferred embodiment, a fusion protein expressed in a retroviral expression vector of the present invention can be used to infect bone marrow cells which are subsequently transplanted into irradiated recipients. The pathology of the subject recipient is then examined after sufficient time has passed (e.g., six (6) weeks).

To maximize recombinant protein expression in *E. coli* is to express the protein in host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, S., *Gene Expression Technology: Methods in Enzymology 185*, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.* (1992) *Nucleic Acids Res.* 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

The NARC8 expression vector can be a yeast expression vector, a vector for expression in insect cells, e.g., a baculovirus expression vector or a vector suitable for expression in mammalian cells.

When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev.* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv. Immunol.* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J.* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) *Proc. Natl. Acad. Sci. USA* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the  $\alpha$ -fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev.* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense



orientation. Regulatory sequences (e.g., viral promoters and/or enhancers) operatively linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the constitutive, tissue specific or cell type specific expression of antisense RNA in a variety of cell types. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus. For a discussion of the regulation of gene expression using antisense genes see Weintraub, H. *et al.* (1986) Antisense RNA as a molecular tool for genetic analysis, *Reviews - Trends in Genetics*, Vol. 1(1).

Another aspect the invention provides a host cell which includes a nucleic acid molecule described herein, e.g., a NARC8 nucleic acid molecule within a recombinant expression vector or a NARC8 nucleic acid molecule containing sequences which allow it to homologously recombine into a specific site of the host cell's genome. The terms "host cell" and "recombinant host cell" are used interchangeably herein. Such terms refer not only to the particular subject cell but rather also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, a NARC8 protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into host cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation.

A host cell of the invention can be used to produce (i.e., express) a NARC8 protein. Accordingly, the invention further provides methods for producing a NARC8 protein using the host cells of the invention. In one embodiment, the method includes culturing the host cell of the invention (into which a recombinant expression vector encoding a NARC8 protein has been introduced) in a suitable medium such that a

NARC8 protein is produced. In another embodiment, the method further includes isolating a NARC8 protein from the medium or the host cell.

In another aspect, the invention features, a cell or purified preparation of cells which include a NARC8 transgene, or which otherwise misexpress NARC8. The cell preparation can consist of human or non-human cells, e.g., rodent cells, e.g., mouse or rat cells, rabbit cells, or pig cells. In preferred embodiments, the cell or cells include a NARC8 transgene, e.g., a heterologous form of a NARC8, e.g., a gene derived from humans (in the case of a non-human cell). The NARC8 transgene can be misexpressed, e.g., overexpressed or underexpressed. In other preferred embodiments, the cell or cells include a gene which misexpress an endogenous NARC8, e.g., a gene the expression of which is disrupted, e.g., a knockout. Such cells can serve as a model for studying disorders which are related to mutated or misexpressed NARC8 alleles or for use in drug screening.

In another aspect, the invention features, a human cell, e.g., a hematopoietic stem cell, transformed with nucleic acid which encodes a subject NARC8 polypeptide.

Also provided are cells or a purified preparation thereof, e.g., human cells, in which an endogenous NARC8 is under the control of a regulatory sequence that does not normally control the expression of the endogenous NARC8 gene. The expression characteristics of an endogenous gene within a cell, e.g., a cell line or microorganism, can be modified by inserting a heterologous DNA regulatory element into the genome of the cell such that the inserted regulatory element is operably linked to the endogenous NARC8 gene. For example, an endogenous NARC8 gene, e.g., a gene which is "transcriptionally silent," e.g., not normally expressed, or expressed only at very low levels, may be activated by inserting a regulatory element which is capable of promoting the expression of a normally expressed gene product in that cell. Techniques such as targeted homologous recombinations, can be used to insert the heterologous DNA as described in, e.g., Chappel, US 5,272,071; WO 91/06667, published on May 16, 1991.

### Transgenic Animals

The invention provides non-human transgenic animals. Such animals are useful for studying the function and/or activity of a NARC8 protein and for identifying and/or

evaluating modulators of NARC8 activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, and the like. A transgene is exogenous DNA or a rearrangement, e.g., a deletion of endogenous chromosomal DNA, which preferably is integrated into or occurs in the genome of the cells of a transgenic animal. A transgene can direct the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal, other transgenes, e.g., a knockout, reduce expression. Thus, a transgenic animal can be one in which an endogenous NARC8 gene has been altered by, e.g., by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to a transgene of the invention to direct expression of a NARC8 protein to particular cells. A transgenic founder animal can be identified based upon the presence of a NARC8 transgene in its genome and/or expression of NARC8 mRNA in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying a transgene encoding a NARC8 protein can further be bred to other transgenic animals carrying other transgenes.

NARC8 proteins or polypeptides can be expressed in transgenic animals or plants, e.g., a nucleic acid encoding the protein or polypeptide can be introduced into the genome of an animal. In preferred embodiments the nucleic acid is placed under the control of a tissue specific promoter, e.g., a milk or egg specific promoter, and recovered from the milk or eggs produced by the animal. Suitable animals are mice, pigs, cows, goats, and sheep.

The invention also includes a population of cells from a transgenic animal, as discussed herein.

## Uses

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenetics); and c) methods of treatment (e.g., therapeutic and prophylactic).

The isolated nucleic acid molecules of the invention can be used, for example, to express a NARC8 protein (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect a NARC8 mRNA (e.g., in a biological sample) or a genetic alteration in a NARC8 gene, and to modulate NARC8 activity, as described further below. The NARC8 proteins can be used to treat disorders characterized by insufficient or excessive production of a NARC8 substrate or production of NARC8 inhibitors. In addition, the NARC8 proteins can be used to screen for naturally occurring NARC8 substrates, to screen for drugs or compounds which modulate NARC8 activity, as well as to treat disorders characterized by insufficient or excessive production of NARC8 protein or production of NARC8 protein forms which have decreased, aberrant or unwanted activity compared to NARC8 wild-type protein. Such disorders include those characterized by aberrant programmed cell death or aberrant cell proliferation. Moreover, the anti-NARC8 antibodies of the invention can be used to detect and isolate NARC8 proteins, regulate the bioavailability of NARC8 proteins, and modulate NARC8 activity.

A method of evaluating a compound for the ability to interact with, e.g., bind, a subject NARC8 polypeptide is provided. The method includes: contacting the compound with the subject NARC8 polypeptide; and evaluating ability of the compound to interact with, e.g., to bind or form a complex with the subject NARC8 polypeptide. This method can be performed *in vitro*, e.g., in a cell free system, or *in vivo*, e.g., in a two-hybrid interaction trap assay. This method can be used to identify naturally occurring molecules which interact with subject NARC8 polypeptide. It can also be used to find natural or synthetic inhibitors of subject NARC8 polypeptide. Screening methods are discussed in more detail below.

### Screening Assays:

The invention provides methods (also referred to herein as "screening assays") for identifying modulators, i.e., candidate or test compounds or agents (e.g., proteins, peptides, peptidomimetics, peptoids, small molecules or other drugs) which bind to NARC8 proteins, have a stimulatory or inhibitory effect on, for example, NARC8 expression or NARC8 activity, or have a stimulatory or inhibitory effect on, for example, the expression or activity of a NARC8 substrate. Compounds thus identified can be used to modulate the activity of target gene products (e.g., NARC8 genes) in a therapeutic protocol, to elaborate the biological function of the target gene product, or to identify compounds that disrupt normal target gene interactions.

In one embodiment, the invention provides assays for screening candidate or test compounds which are substrates of a NARC8 protein or polypeptide or a biologically active portion thereof. In another embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of a NARC8 protein or polypeptide or a biologically active portion thereof.

The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; peptoid libraries [libraries of molecules having the functionalities of peptides, but with a novel, non-peptide backbone which are resistant to enzymatic degradation but which nevertheless remain bioactive] (see, e.g., Zuckermann, R.N. *et al.* (1994) *J. Med. Chem.* 37:2678-85); spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The biological library and peptoid library approaches are limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994). *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed.*

*Engl.* 33:2059; Carell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria or spores (Ladner, United States Patent No. 5,223,409), plasmids (Cull *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310); (Ladner *supra.*).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a NARC8 protein or biologically active portion thereof is contacted with a test compound, and the ability of the test compound to modulate NARC8 activity is determined. Determining the ability of the test compound to modulate NARC8 activity can be accomplished by monitoring, for example, programmed cell death-associated protein activity. The cell, for example, can be of mammalian origin, e.g., human. Cell homogenates, or fractions, preferably membrane containing fractions, can also be tested.

The ability of the test compound to modulate NARC8 binding to a compound, e.g., a NARC8 substrate, or to bind to NARC8 can also be evaluated. This can be accomplished, for example, by coupling the compound, e.g., the substrate, with a radioisotope or enzymatic label such that binding of the compound, e.g., the substrate, to NARC8 can be determined by detecting the labeled compound, e.g., substrate, in a complex. Alternatively, NARC8 could be coupled with a radioisotope or enzymatic label to monitor the ability of a test compound to modulate NARC8 binding to a NARC8 substrate in a complex. For example, compounds (e.g., NARC8 substrates) can be labeled with  $^{125}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ , or  $^3\text{H}$ , either directly or indirectly, and the radioisotope detected by direct counting of radioemission or by scintillation counting. Alternatively, compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product.

The ability of a compound (e.g., a NARC8 substrate) to interact with NARC8 with or without the labeling of any of the interactants can be evaluated. For example, a

microphysiometer can be used to detect the interaction of a compound with NARC8 without the labeling of either the compound or the NARC8. McConnell, H. M. *et al.* (1992) *Science* 257:1906-1912. As used herein, a "microphysiometer" (e.g., Cytosensor) is an analytical instrument that measures the rate at which a cell acidifies its environment using a light-addressable potentiometric sensor (LAPS). Changes in this acidification rate can be used as an indicator of the interaction between a compound and NARC8.

In yet another embodiment, a cell-free assay is provided in which a NARC8 protein or biologically active portion thereof is contacted with a test compound and the ability of the test compound to bind to the NARC8 protein or biologically active portion thereof is evaluated. Preferred biologically active portions of the NARC8 proteins to be used in assays of the present invention include fragments which participate in interactions with non-NARC8 molecules, e.g., fragments with high surface probability scores.

Soluble and/or membrane-bound forms of isolated proteins (e.g., NARC8 proteins or biologically active portions thereof) can be used in the cell-free assays of the invention. When membrane-bound forms of the protein are used, it may be desirable to utilize a solubilizing agent. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-dodecylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton<sup>®</sup> X-100, Triton<sup>®</sup> X-114, Thesit<sup>®</sup>, Isotridecypoly(ethylene glycol ether)<sub>n</sub>, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl-N,N-dimethyl-3-ammonio-1-propane sulfonate.

Cell-free assays involve preparing a reaction mixture of the target gene protein and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex that can be removed and/or detected.

In one embodiment, assays are performed where the ability of an agent to block programmed cell death-associated protein activity within a cell is evaluated.

The interaction between two molecules can also be detected, e.g., using fluorescence energy transfer (FET) (see, for example, Lakowicz *et al.* U.S. Patent No.

5,631,169; Stavrianopoulos, *et al.* U.S. Patent No. 4,868,103). A fluorophore label on the first, 'donor' molecule is selected such that its emitted fluorescent energy will be absorbed by a fluorescent label on a second, 'acceptor' molecule, which in turn is able to fluoresce due to the absorbed energy. Alternately, the 'donor' protein molecule may simply utilize the natural fluorescent energy of tryptophan residues. Labels are chosen that emit different wavelengths of light, such that the 'acceptor' molecule label may be differentiated from that of the 'donor'. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. An FET binding event can be conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter).

In another embodiment, determining the ability of the NARC8 protein to bind to a target molecule can be accomplished using real-time Biomolecular Interaction Analysis (BIA) (see, e.g., Sjolander, S. and Urbaniczky, C. (1991) *Anal. Chem.* 63:2338-2345 and Szabo *et al.* (1995) *Curr. Opin. Struct. Biol.* 5:699-705). "Surface plasmon resonance" or "BIA" detects biospecific interactions in real time, without labeling any of the interactants (e.g., BIAcore). Changes in the mass at the binding surface (indicative of a binding event) result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)), resulting in a detectable signal which can be used as an indication of real-time reactions between biological molecules.

In one embodiment, the target gene product or the test substance is anchored onto a solid phase. The target gene product/test compound complexes anchored on the solid phase can be detected at the end of the reaction. Preferably, the target gene product can be anchored onto a solid surface, and the test compound, (which is not anchored), can be labeled, either directly or indirectly, with detectable labels discussed herein.

It may be desirable to immobilize either NARC8, an anti-NARC8 antibody or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a test compound to a NARC8 protein, or interaction of a NARC8 protein with a target molecule in the presence and absence of a candidate compound, can be accomplished in



any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase/NARC8 fusion proteins or

5 glutathione-S-transferase/target fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, MO) or glutathione derivatized microtiter plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or NARC8 protein, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and

10 pH). Following incubation, the beads or microtiter plate wells are washed to remove any unbound components, the matrix immobilized in the case of beads, complex determined either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of NARC8 binding or activity determined using standard techniques.

15 Other techniques for immobilizing either a NARC8 protein or a target molecule on matrices include using conjugation of biotin and streptavidin. Biotinylated NARC8 protein or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques known in the art (e.g., biotinylation kit, Pierce Chemicals, Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical).

20 In order to conduct the assay, the non-immobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways.

25 Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly

30 labeled with, e.g., a labeled anti-Ig antibody).

In one embodiment, this assay is performed utilizing antibodies reactive with NARC8 protein or target molecules but which do not interfere with binding of the NARC8 protein to its target molecule. Such antibodies can be derivatized to the wells of the plate, and unbound target or NARC8 protein trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the NARC8 protein or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the NARC8 protein or target molecule.

Alternatively, cell free assays can be conducted in a liquid phase. In such an assay, the reaction products are separated from unreacted components, by any of a number of standard techniques, including but not limited to: differential centrifugation (see, for example, Rivas, G., and Minton, A.P. (1993 Aug) *Trends Biochem Sci* 18(8):284-7); chromatography (gel filtration chromatography, ion-exchange chromatography); electrophoresis (see, e.g., Ausubel, F. *et al.* eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York.); and immunoprecipitation (see, for example, Ausubel, F. *et al.* eds. *Current Protocols in Molecular Biology* 1999, J. Wiley: New York). Such resins and chromatographic techniques are known to one skilled in the art (see, e.g., Heegaard, N.H. (1998 Winter) *J Mol. Recognit.* 11(1-6):141-8; Hage, D.S., and Tweed, S.A. (1997, Oct 10) *J. Chromatogr. B Biomed. Sci. Appl.* 699(1-2):499-525). Further, fluorescence energy transfer may also be conveniently utilized, as described herein, to detect binding without further purification of the complex from solution.

In a preferred embodiment, the assay includes contacting the NARC8 protein or biologically active portion thereof with a known compound which binds NARC8 to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with a NARC8 protein, wherein determining the ability of the test compound to interact with a NARC8 protein includes determining the ability of the test compound to preferentially bind to NARC8 or biologically active portion thereof, or to modulate the activity of a target molecule, as compared to the known compound.

The target gene products of the invention can, *in vivo*, interact with one or more cellular or extracellular macromolecules, such as proteins. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partners." Compounds that disrupt such interactions can be useful in regulating the activity of the target gene product. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and small molecules. The preferred target genes/products for use in this embodiment are the NARC8 genes herein identified. In an alternative embodiment, the invention provides methods for determining the ability of the test compound to modulate the activity of a NARC8 protein through modulation of the activity of a downstream effector of a NARC8 target molecule. For example, the activity of the effector molecule on an appropriate target can be determined, or the binding of the effector to an appropriate target can be determined, as previously described.

To identify compounds that interfere with the interaction between the target gene product and its cellular or extracellular binding partner(s), e.g., a substrate, a reaction mixture containing the target gene product and the binding partner is prepared, under conditions and for a time sufficient, to allow the two products to form complex. In order to test an inhibitory agent, the reaction mixture is provided in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of the target gene and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the target gene product and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the target gene product and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal target gene product can also be compared to complex formation within reaction mixtures containing the test compound and mutant target gene product. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal target gene products.

These assays can be conducted in a heterogeneous or homogeneous format.

Heterogeneous assays involve anchoring either the target gene product or the binding partner onto a solid phase, and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the target gene products and the binding partners, e.g., by competition, can be identified by conducting the reaction in the presence of the test substance. Alternatively, test compounds that disrupt preformed complexes, e.g., compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are briefly described below.

In a heterogeneous assay system, either the target gene product or the interactive cellular or extracellular binding partner, is anchored onto a solid surface (e.g., a microtiter plate), while the non-anchored species is labeled, either directly or indirectly. The anchored species can be immobilized by non-covalent or covalent attachments. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with, e.g., a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds that inhibit complex formation or that disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted

components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds that inhibit complex or that disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. For example, a preformed complex of the target gene product and the interactive cellular or extracellular binding partner product is prepared in that either the target gene products or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Patent No. 4,109,496 that utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances that disrupt target gene product-binding partner interaction can be identified.

In yet another aspect, the NARC8 proteins can be used as "bait proteins" in a two-hybrid assay or three-hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos *et al.* (1993) *Cell* 72:223-232; Madura *et al.* (1993) *J. Biol. Chem.* 268:12046-12054; Bartel *et al.* (1993) *Biotechniques* 14:920-924; Iwabuchi *et al.* (1993) *Oncogene* 8:1693-1696; and Brent WO94/10300), to identify other proteins, which bind to or interact with NARC8 ("NARC8-binding proteins" or "NARC8-bp") and are involved in NARC8 activity. Such NARC8-bps can be activators or inhibitors of signals by the NARC8 proteins or NARC8 targets as, for example, downstream elements of a NARC8-mediated signaling pathway.

The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. In one construct, the gene that codes for a NARC8 protein is fused to a gene encoding the DNA binding domain of a known transcription factor (e.g., GAL-4). In the other construct, a DNA sequence, from a library of DNA sequences, that encodes an unidentified protein ("prey" or "sample") is fused to a gene that codes for the activation domain of the known transcription factor.

(Alternatively the: NARC8 protein can be the fused to the activator domain.) If the "bait" and the "prey" proteins are able to interact, *in vivo*, forming a NARC8-dependent

complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ) which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected and cell colonies containing the functional transcription factor can be isolated and used to obtain the cloned gene which encodes the protein which interacts with the NARC8 protein.

In another embodiment, modulators of NARC8 expression are identified. For example, a cell or cell free mixture is contacted with a candidate compound and the expression of NARC8 mRNA or protein evaluated relative to the level of expression of NARC8 mRNA or protein in the absence of the candidate compound. When expression of NARC8 mRNA or protein is greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of NARC8 mRNA or protein expression. Alternatively, when expression of NARC8 mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of NARC8 mRNA or protein expression. The level of NARC8 mRNA or protein expression can be determined by methods described herein for detecting NARC8 mRNA or protein.

In another aspect, the invention pertains to a combination of two or more of the assays described herein. For example, a modulating agent can be identified using a cell-based or a cell free assay, and the ability of the agent to modulate the activity of a NARC8 protein can be confirmed *in vivo*, e.g., in an animal.

This invention further pertains to novel agents identified by the above-described screening assays. Accordingly, it is within the scope of this invention to further use an agent identified as described herein (e.g., a NARC8 modulating agent, an antisense NARC8 nucleic acid molecule, a NARC8-specific antibody, or a NARC8-binding partner) in an appropriate animal model to determine the efficacy, toxicity, side effects, or mechanism of action, of treatment with such an agent. Furthermore, novel agents identified by the above-described screening assays can be used for treatments as described herein.

## Detection Assays

Portions or fragments of the nucleic acid sequences identified herein can be used as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome e.g., to locate gene regions associated with genetic disease or to associate NARC8 with a disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

## Chromosome Mapping

The NARC8 nucleotide sequences or portions thereof can be used to map the location of the NARC8 genes on a chromosome. This process is called chromosome mapping. Chromosome mapping is useful in correlating the NARC8 sequences with genes associated with disease.

Briefly, NARC8 genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the NARC8 nucleotide sequences. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the NARC8 sequences will yield an amplified fragment.

A panel of somatic cell hybrids in which each cell line contains either a single human chromosome or a small number of human chromosomes, and a full set of mouse chromosomes, can allow easy mapping of individual genes to specific human chromosomes. (D'Eustachio P. *et al.* (1983) *Science* 220:919-924).

Other mapping strategies e.g., in situ hybridization (described in Fan, Y. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:6223-27), pre-screening with labeled flow-sorted chromosomes, and pre-selection by hybridization to chromosome specific cDNA libraries can be used to map NARC8 to a chromosomal location.

Fluorescence *in situ* hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. The FISH technique can be used with a DNA sequence as short as 500 or 600 bases. However, clones larger than 1,000 bases have a higher likelihood of binding to a unique chromosomal location with sufficient signal intensity for simple detection.

Preferably 1,000 bases, and more preferably 2,000 bases will suffice to get good results at a reasonable amount of time. For a review of this technique, see Verma *et al.* *Human Chromosomes: A Manual of Basic Techniques* (Pergamon Press, New York 1988).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between a gene and a disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, for example, Egeland, J. *et al.* (1987) *Nature* 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with the NARC8 gene, can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes, such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

### Tissue Typing

NARC8 sequences can be used to identify individuals from biological samples using, e.g., restriction fragment length polymorphism (RFLP). In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, the fragments separated, e.g., in a Southern blot, and probed to yield bands for identification.



The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can also be used to determine the actual base-by-base DNA sequence of selected portions of an individual's genome.

5 Thus, the NARC8 nucleotide sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it. Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to  
10 allelic differences.

Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur  
15 in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1 or SEQ ID NO:4 can provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:3 or SEQ ID NO:6 are used, a more appropriate number of primers for positive  
20 individual identification would be 500-2,000.

If a panel of reagents from NARC8 nucleotide sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from  
25 extremely small tissue samples.

#### Use of Partial NARC8 Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. To make such an identification, PCR technology can be used to amplify DNA sequences  
30 taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be

compared to a standard, thereby allowing identification of the origin of the biological sample.

5 The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions of SEQ ID NO:1 or SEQ  
10 ID NO:4 (e.g., fragments derived from the noncoding regions of SEQ ID NO:1 or SEQ ID NO:4 having a length of at least 20 bases, preferably at least 30 bases) are particularly appropriate for this use.

The NARC8 nucleotide sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for  
15 example, an *in situ* hybridization technique, to identify a specific tissue, e.g., a tissue containing programmed cell death-associated protein activity. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such NARC8 probes can be used to identify tissue by species and/or by organ type.

In a similar fashion, these reagents, e.g., NARC8 primers or probes can be used to  
20 screen tissue culture for contamination (i.e. screen for the presence of a mixture of different types of cells in a culture).

### Predictive Medicine

The present invention also pertains to the field of predictive medicine in which  
25 diagnostic assays, prognostic assays, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual.

Generally, the invention provides, a method of determining if a subject is at risk for a disorder related to a lesion in or the misexpression of a gene which encodes NARC8.

30 Such disorders include, e.g., a disorder associated with the misexpression of NARC8, or lipid metabolism related disorder.

The method includes one or more of the following:

detecting, in a tissue of the subject, the presence or absence of a mutation which affects the expression of the NARC8 gene, or detecting the presence or absence of a mutation in a region which controls the expression of the gene, e.g., a mutation in the 5'

5 control region;

detecting, in a tissue of the subject, the presence or absence of a mutation which alters the structure of the NARC8 gene;

detecting, in a tissue of the subject, the misexpression of the NARC8 gene, at the mRNA level, e.g., detecting a non-wild type level of a mRNA ;

10 detecting, in a tissue of the subject, the misexpression of the gene, at the protein level, e.g., detecting a non-wild type level of a NARC8 polypeptide.

In preferred embodiments the method includes: ascertaining the existence of at least one of: a deletion of one or more nucleotides from the NARC8 gene; an insertion of one or more nucleotides into the gene, a point mutation, e.g., a substitution of one or more nucleotides of the gene, a gross chromosomal rearrangement of the gene, e.g., a translocation, inversion, or deletion.

For example, detecting the genetic lesion can include: (i) providing a probe/primer including an oligonucleotide containing a region of nucleotide sequence which hybridizes to a sense or antisense sequence from SEQ ID NO:1 or SEQ ID NO:4 naturally occurring mutants thereof or 5' or 3' flanking sequences naturally associated with the NARC8 gene; (ii) exposing the probe/primer to nucleic acid of the tissue; and detecting, by hybridization, e.g., *in situ* hybridization, of the probe/primer to the nucleic acid, the presence or absence of the genetic lesion.

In preferred embodiments detecting the misexpression includes ascertaining the existence of at least one of: an alteration in the level of a messenger RNA transcript of the NARC8 gene; the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene; or a non-wild type level of NARC8.

Methods of the invention can be used prenatally or to determine if a subject's offspring will be at risk for a disorder.

30 In preferred embodiments the method includes determining the structure of a NARC8 gene, an abnormal structure being indicative of risk for the disorder.

In preferred embodiments the method includes contacting a sample from the subject with an antibody to the NARC8 protein or a nucleic acid, which hybridizes specifically with the gene. These and other embodiments are discussed below.

## 5 Diagnostic and Prognostic Assays

The presence, level, or absence of NARC8 protein or nucleic acid in a biological sample can be evaluated by obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting NARC8 protein or nucleic acid (e.g., mRNA, genomic DNA) that encodes NARC8 protein such that the presence of NARC8 protein or nucleic acid is detected in the biological sample. The term "biological sample" includes tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. A preferred biological sample is serum. The level of expression of the NARC8 gene can be measured in a number of ways, including, but not limited to: measuring the mRNA encoded by the NARC8 genes; measuring the amount of protein encoded by the NARC8 genes; or measuring the activity of the protein encoded by the NARC8 genes.

The level of mRNA corresponding to the NARC8 gene in a cell can be determined both by *in situ* and by *in vitro* formats.

The isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or Northern analyses, polymerase chain reaction analyses and probe arrays. One preferred diagnostic method for the detection of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to the mRNA encoded by the gene being detected. The nucleic acid probe can be, for example, a full-length NARC8 nucleic acid, such as the nucleic acid of SEQ ID NO:1, SEQ ID NO:4, or the DNA insert of the plasmid deposited with ATCC as Accession Number \_\_\_\_\_ or \_\_\_\_\_, or a portion thereof, such as an oligonucleotide of at least 7, 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to NARC8 mRNA or genomic DNA. Other suitable probes for use in the diagnostic assays are described herein.

In one format, mRNA (or cDNA) is immobilized on a surface and contacted with the probes, for example by running the isolated mRNA on an agarose gel and transferring

the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative format, the probes are immobilized on a surface and the mRNA (or cDNA) is contacted with the probes, for example, in a two-dimensional gene chip array. A skilled artisan can adapt known mRNA detection methods for use in detecting the level of mRNA encoded by the

5 NARC8 genes.

The level of mRNA in a sample that is encoded by one of NARC8 can be evaluated with nucleic acid amplification, e.g., by rtPCR (Mullis, 1987, U.S. Patent No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 10 87:1874-1878), transcriptional amplification system (Kwoh *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi *et al.* (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi *et al.* U.S. Patent No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques known in the art. As used herein, amplification primers are defined as  
15 being a pair of nucleic acid molecules that can anneal to 5' or 3' regions of a gene (plus and minus strands, respectively, or vice-versa) and contain a short region in between. In general, amplification primers are from about 10 to 30 nucleotides in length and flank a region from about 50 to 200 nucleotides in length. Under appropriate conditions and with appropriate reagents, such primers permit the amplification of a nucleic acid  
20 molecule comprising the nucleotide sequence flanked by the primers.

For *in situ* methods, a cell or tissue sample can be prepared/processed and immobilized on a support, typically a glass slide, and then contacted with a probe that can hybridize to mRNA that encodes the NARC8 gene being analyzed.

In another embodiment, the methods further contacting a control sample with a  
25 compound or agent capable of detecting NARC8 mRNA, or genomic DNA, and comparing the presence of NARC8 mRNA or genomic DNA in the control sample with the presence of NARC8 mRNA or genomic DNA in the test sample.

A variety of methods can be used to determine the level of protein encoded by NARC8. In general, these methods include contacting an agent that selectively binds to  
30 the protein, such as an antibody with a sample, to evaluate the level of protein in the sample. In a preferred embodiment, the antibody bears a detectable label. Antibodies

can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with a detectable substance. Examples of detectable substances are provided herein.

The detection methods can be used to detect NARC8 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of NARC8 protein include enzyme linked immunosorbent assays (ELISAs), immunoprecipitations, immunofluorescence, enzyme immunoassay (EIA), radioimmunoassay (RIA), and Western blot analysis. *In vivo* techniques for detection of NARC8 protein include introducing into a subject a labeled anti-NARC8 antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In another embodiment, the methods further include contacting the control sample with a compound or agent capable of detecting NARC8 protein, and comparing the presence of NARC8 protein in the control sample with the presence of NARC8 protein in the test sample.

The invention also includes kits for detecting the presence of NARC8 in a biological sample. For example, the kit can include a compound or agent capable of detecting NARC8 protein or mRNA in a biological sample; and a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect NARC8 protein or nucleic acid.

For antibody-based kits, the kit can include: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide corresponding to a marker of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can include: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide corresponding to a marker of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule corresponding to a marker of the invention. The

kit can also includes a buffering agent, a preservative, or a protein-stabilizing agent. The kit can also includes components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each  
5 component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit.

The diagnostic methods described herein can identify subjects having, or at risk of developing, a disease or disorder associated with misexpressed or aberrant or unwanted  
10 NARC8 expression or activity. As used herein, the term "unwanted" includes an unwanted phenomenon involved in a biological response such as inappropriately high levels of programmed cell death.

In one embodiment, a disease or disorder associated with aberrant or unwanted NARC8 expression or activity is identified. A test sample is obtained from a subject and  
15 NARC8 protein or nucleic acid (e.g., mRNA or genomic DNA) is evaluated, wherein the level, e.g., the presence or absence, of NARC8 protein or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant or unwanted NARC8 expression or activity. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest, including a biological fluid (e.g.,  
20 serum), cell sample, or tissue.

The prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant or unwanted NARC8 expression or activity. For  
25 example, such methods can be used to determine whether a subject can be effectively treated with an agent for modulating programmed cell death.

The methods of the invention can also be used to detect genetic alterations in a NARC8 gene, thereby determining if a subject with the altered gene is at risk for a disorder characterized by misregulation in NARC8 protein activity or nucleic acid  
30 expression, such as a programmed cell death related disorder. In preferred embodiments, the methods include detecting, in a sample from the subject, the presence or absence of a

genetic alteration characterized by at least one of an alteration affecting the integrity of a gene encoding a NARC8-protein, or the misexpression of the NARC8 gene. For example, such genetic alterations can be detected by ascertaining the existence of at least one of 1) a deletion of one or more nucleotides from a NARC8 gene; 2) an addition of one or more nucleotides to a NARC8 gene; 3) a substitution of one or more nucleotides of a NARC8 gene, 4) a chromosomal rearrangement of a NARC8 gene; 5) an alteration in the level of a messenger RNA transcript of a NARC8 gene, 6) aberrant modification of a NARC8 gene, such as of the methylation pattern of the genomic DNA, 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of a NARC8 gene, 8) a non-wild type level of a NARC8-protein, 9) allelic loss of a NARC8 gene, and 10) inappropriate post-translational modification of a NARC8-protein.

An alteration can be detected without a probe/primer in a polymerase chain reaction, such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR), the latter of which can be particularly useful for detecting point mutations in the NARC8-gene. This method can include the steps of collecting a sample of cells from a subject, isolating nucleic acid (e.g., genomic, mRNA or both) from the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a NARC8 gene under conditions such that hybridization and amplification of the NARC8-gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli, J.C. *et al.* (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh, D.Y. *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi, P.M. *et al.* (1988) *Bio-Technology* 6:1197), or other nucleic acid amplification methods, followed by the detection of the amplified molecules using techniques known to those of skill in the art.

In another embodiment, mutations in a NARC8 gene from a sample cell can be identified by detecting alterations in restriction enzyme cleavage patterns. For example,



sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined, e.g., by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific ribozymes (see, for example, U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations in NARC8 can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, two-dimensional arrays, e.g., chip based arrays. Such arrays include a plurality of addresses, each of which is positionally distinguishable from the other. A different probe is located at each address of the plurality. The arrays can have a high density of addresses, e.g., can contain hundreds or thousands of oligonucleotides probes (Cronin, M.T. *et al.* (1996) *Human Mutation* 7: 244-255; Kozal, M.J. *et al.* (1996) *Nature Medicine* 2:753-759). For example, genetic mutations in NARC8 can be identified in two dimensional arrays containing light-generated DNA probes as described in Cronin, M.T. *et al. supra*. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the NARC8 gene and detect mutations by comparing the sequence of the sample NARC8 with the corresponding wild-type (control) sequence. Automated sequencing procedures can be utilized when performing the diagnostic assays (Naeve *et al.* (1995) *Biotechniques* 19:448-453), including sequencing by mass spectrometry.

Other methods for detecting mutations in the NARC8 gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA

or RNA/DNA heteroduplexes (Myers *et al.* (1985) *Science* 230:1242-1246; Cotton *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4397-4401; Saleeba *et al.* (1992) *Methods Enzymol.* 217:286-295).

5 In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in NARC8 cDNAs obtained from samples of cells. For example, the mutY enzyme of *E. coli* cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu *et al.* (1994) *Carcinogenesis* 15:1657-1662; U.S. Patent No. 5,459,039).

10 In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in NARC8 genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita *et al.* (1989) *Proc. Natl. Acad. Sci. USA*: 86:2766-2770, see also Cotton (1993) *Mutat. Res.* 285:125-144; and Hayashi (1992) *Genet. Anal. Tech. Appl.* 9:73-79). Single-stranded DNA fragments of sample and control NARC8 nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility

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25 (Keen *et al.* (1991) *Trends Genet.* 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers *et al.* (1985) *Nature* 313:495-498). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used

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in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) *Biophys. Chem.* 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension (Saiki *et al.* (1986) *Nature* 324:163); Saiki *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:6230).

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention.

Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs *et al.* (1989) *Nucleic Acids Res.* 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent, or reduce polymerase extension (Prossner (1993) *Tibtech* 11:238). In addition it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini *et al.* (1992) *Mol. Cell Probes* 6:1-7). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) *Proc. Natl. Acad. Sci USA* 88:189-193). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, *e.g.*, in clinical settings to diagnose patients exhibiting symptoms or family history of a disease or illness involving a NARC8 gene.

#### Use of NARC8 Molecules as Surrogate Markers

The NARC8 molecules of the invention are also useful as markers of disorders or disease states, as markers for precursors of disease states, as markers for predisposition of disease states, as markers of drug activity, or as markers of the pharmacogenomic profile of a subject. Using the methods described herein, the presence, absence and/or quantity

of the NARC8 molecules of the invention may be detected, and may be correlated with one or more biological states *in vivo*. For example, the NARC8 molecules of the invention may serve as surrogate markers for one or more disorders or disease states or for conditions leading up to disease states. As used herein, a “surrogate marker” is an objective biochemical marker which correlates with the absence or presence of a disease or disorder, or with the progression of a disease or disorder (*e.g.*, with the presence or absence of a tumor). The presence or quantity of such markers is independent of the disease. Therefore, these markers may serve to indicate whether a particular course of treatment is effective in lessening a disease state or disorder. Surrogate markers are of particular use when the presence or extent of a disease state or disorder is difficult to assess through standard methodologies (*e.g.*, early stage tumors), or when an assessment of disease progression is desired before a potentially dangerous clinical endpoint is reached (*e.g.*, an assessment of cardiovascular disease may be made using cholesterol levels as a surrogate marker, and an analysis of HIV infection may be made using HIV RNA levels as a surrogate marker, well in advance of the undesirable clinical outcomes of myocardial infarction or fully-developed AIDS). Examples of the use of surrogate markers in the art include: Koomen *et al.* (2000) *J. Mass. Spectrom.* 35: 258-264; and James (1994) *AIDS Treatment News Archive* 209.

The NARC8 molecules of the invention are also useful as pharmacodynamic markers. As used herein, a “pharmacodynamic marker” is an objective biochemical marker which correlates specifically with drug effects. The presence or quantity of a pharmacodynamic marker is not related to the disease state or disorder for which the drug is being administered; therefore, the presence or quantity of the marker is indicative of the presence or activity of the drug in a subject. For example, a pharmacodynamic marker may be indicative of the concentration of the drug in a biological tissue, in that the marker is either expressed or transcribed or not expressed or transcribed in that tissue in relationship to the level of the drug. In this fashion, the distribution or uptake of the drug may be monitored by the pharmacodynamic marker. Similarly, the presence or quantity of the pharmacodynamic marker may be related to the presence or quantity of the metabolic product of a drug, such that the presence or quantity of the marker is indicative of the relative breakdown rate of the drug *in vivo*. Pharmacodynamic markers

are of particular use in increasing the sensitivity of detection of drug effects, particularly when the drug is administered in low doses. Since even a small amount of a drug may be sufficient to activate multiple rounds of marker (e.g., a NARC8 marker) transcription or expression, the amplified marker may be in a quantity which is more readily detectable than the drug itself. Also, the marker may be more easily detected due to the nature of the marker itself; for example, using the methods described herein, anti-NARC8 antibodies may be employed in an immune-based detection system for a NARC8 protein marker, or NARC8-specific radiolabeled probes may be used to detect a NARC8 mRNA marker. Furthermore, the use of a pharmacodynamic marker may offer mechanism-based prediction of risk due to drug treatment beyond the range of possible direct observations. Examples of the use of pharmacodynamic markers in the art include: Matsuda *et al.* US 6,033,862; Hattis *et al.* (1991) *Env. Health Perspect.* 90:229-238; Schentag (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl.3:S21-S24; and Nicolau (1999) *Am. J. Health-Syst. Pharm.* 56 Suppl.3:S16-S20.

The NARC8 molecules of the invention are also useful as pharmacogenomic markers. As used herein, a "pharmacogenomic marker" is an objective biochemical marker which correlates with a specific clinical drug response or susceptibility in a subject (see, e.g., McLeod *et al.* (1999) *Eur. J. Cancer* 35(12): 1650-1652). The presence or quantity of the pharmacogenomic marker is related to the predicted response of the subject to a specific drug or class of drugs prior to administration of the drug. By assessing the presence or quantity of one or more pharmacogenomic markers in a subject, a drug therapy which is most appropriate for the subject, or which is predicted to have a greater degree of success, may be selected. For example, based on the presence or quantity of RNA, or protein (e.g., NARC8 protein or RNA) for specific tumor markers in a subject, a drug or course of treatment may be selected that is optimized for the treatment of the specific tumor likely to be present in the subject. Similarly, the presence or absence of a specific sequence mutation in NARC8 DNA may correlate NARC8 drug response. The use of pharmacogenomic markers therefore permits the application of the most appropriate treatment for each subject without having to administer the therapy.

## Pharmaceutical Compositions

The nucleic acid and polypeptides, fragments thereof, as well as anti-NARC8 antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions. Such compositions typically include the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of  
5 surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be  
10 brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions  
15 are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously  
20 sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash.  
25 Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant  
30 such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a

sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant,  
5 e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and  
10 fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with  
15 conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems.  
20 Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected  
25 cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used  
30 herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to



produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for  
5 determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system  
10 that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or  
15 no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (i.e., the concentration of the test  
20 compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

As defined herein, a therapeutically effective amount of protein or polypeptide  
25 (i.e., an effective dosage) ranges from about 0.001 to 30 mg/kg body weight, preferably about 0.01 to 25 mg/kg body weight, more preferably about 0.1 to 20 mg/kg body weight, and even more preferably about 1 to 10 mg/kg, 2 to 9 mg/kg, 3 to 8 mg/kg, 4 to 7 mg/kg, or 5 to 6 mg/kg body weight. The protein or polypeptide can be administered one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more  
30 preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. The skilled artisan will appreciate that certain factors may influence the dosage

and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a protein, polypeptide, or antibody can include a single treatment or, preferably, can include a series of treatments.

For antibodies, the preferred dosage is 0.1 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies.

Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank *et al.* ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The present invention encompasses agents which modulate expression or activity. An agent may, for example, be a small molecule. For example, such small molecules include, but are not limited to, peptides, peptidomimetics (e.g., peptoids), amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

Exemplary doses include milligram or microgram amounts of the small molecule per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram).

It is furthermore understood that appropriate doses of a small molecule depend upon the potency of the small molecule with respect to the expression or activity to be modulated.

When one or more of these small molecules is to be administered to an animal (e.g., a human) in order to modulate expression or activity of a polypeptide or nucleic acid of the invention, a physician, veterinarian, or researcher may, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

10           An antibody (or fragment thereof) may be conjugated to a therapeutic moiety such as a cytotoxin, a therapeutic agent or a radioactive metal ion. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy  
15   anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan,  
20   carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and  
25   vinblastine).

          The conjugates of the invention can be used for modifying a given biological response, the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin  
30   such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, .alpha.-interferon, .beta.-interferon, nerve growth factor, platelet

derived growth factor, tissue plasminogen activator; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophase colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

5           Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

          The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by  
10       stereotactic injection (see e.g., Chen *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery  
15       vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

          The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

## 20       Methods of Treatment:

          The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant or unwanted NARC8 expression or activity. With regards to both prophylactic and therapeutic methods of treatment, such treatments may be specifically  
25       tailored or modified, based on knowledge obtained from the field of pharmacogenomics. "Pharmacogenomics", as used herein, refers to the application of genomics technologies such as gene sequencing, statistical genetics, and gene expression analysis to drugs in clinical development and on the market. More specifically, the term refers the study of how a patient's genes determine his or her response to a drug (e.g., a patient's "drug  
30       response phenotype", or "drug response genotype".) Thus, another aspect of the invention provides methods for tailoring an individual's prophylactic or therapeutic

treatment with either the NARC8 molecules of the present invention or NARC8 modulators according to that individual's drug response genotype. Pharmacogenomics allows a clinician or physician to target prophylactic or therapeutic treatments to patients who will most benefit from the treatment and to avoid treatment of patients who will experience toxic drug-related side effects.

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant or unwanted NARC8 expression or activity, by administering to the subject a NARC8 or an agent which modulates NARC8 expression or at least one NARC8 activity. Subjects at risk for a disease which is caused or contributed to by aberrant or unwanted NARC8 expression or activity can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the NARC8 aberrance, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of NARC8 aberrance, for example, a NARC8, NARC8 agonist or NARC8 antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

It is possible that some NARC8 disorders can be caused, at least in part, by an abnormal level of gene product, or by the presence of a gene product exhibiting abnormal activity. As such, the reduction in the level and/or activity of such gene products would bring about the amelioration of disorder symptoms.

As discussed, successful treatment of NARC8 disorders can be brought about by techniques that serve to inhibit the expression or activity of target gene products. For example, compounds, e.g., an agent identified using an assays described above, that proves to exhibit negative modulatory activity, can be used in accordance with the invention to prevent and/or ameliorate symptoms of NARC8 disorders. Such molecules can include, but are not limited to peptides, phosphopeptides, small organic or inorganic molecules, or antibodies (including, for example, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')<sub>2</sub> and FAb expression library fragments, scFV molecules, and epitope-binding fragments thereof).

Further, antisense and ribozyme molecules that inhibit expression of the target gene can also be used in accordance with the invention to reduce the level of target gene expression, thus effectively reducing the level of target gene activity. Still further, triple helix molecules can be utilized in reducing the level of target gene activity. Antisense, ribozyme and triple helix molecules are discussed above.

It is possible that the use of antisense, ribozyme, and/or triple helix molecules to reduce or inhibit mutant gene expression can also reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles, such that the concentration of normal target gene product present can be lower than is necessary for a normal phenotype. In such cases, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity can be introduced into cells via gene therapy method. Alternatively, in instances in that the target gene encodes an extracellular protein, it can be preferable to co-administer normal target gene protein into the cell or tissue in order to maintain the requisite level of cellular or tissue target gene activity.

Another method by which nucleic acid molecules may be utilized in treating or preventing a disease characterized by NARC8 expression is through the use of aptamer molecules specific for NARC8 protein. Aptamers are nucleic acid molecules having a tertiary structure which permits them to specifically bind to protein ligands (see, e.g., Osborne, *et al.* (1997) *Curr. Opin. Chem. Biol.* 1(1):5-9; and Patel, D.J. (1997 Jun) *Curr. Opin. Chem. Biol.* 1(1):32-46). Since nucleic acid molecules may in many cases be more conveniently introduced into target cells than therapeutic protein molecules may be, aptamers offer a method by which NARC8 protein activity may be specifically decreased without the introduction of drugs or other molecules which may have pluripotent effects.

Antibodies can be generated that are both specific for target gene product and that reduce target gene product activity. Such antibodies may, therefore, be administered in instances whereby negative modulatory techniques are appropriate for the treatment of NARC8 disorders. For a description of antibodies, see the Antibody section above.

In circumstances wherein injection of an animal or a human subject with a NARC8 protein or epitope for stimulating antibody production is harmful to the subject, it is possible to generate an immune response against NARC8 through the use of anti-

idiotypic antibodies (see, for example, Herlyn, D. (1999) *Ann. Med.* 31(1):66-78; and  
Bhattacharya-Chatterjee, M., and Foon, K.A. (1998) *Cancer Treat. Res.* 94:51-68). If an  
anti-idiotypic antibody is introduced into a mammal or human subject, it should stimulate  
the production of anti-anti-idiotypic antibodies, which should be specific to the NARC8  
5 protein. Vaccines directed to a disease characterized by NARC8 expression may also be  
generated in this fashion.

In instances where the target antigen is intracellular and whole antibodies are  
used, internalizing antibodies may be preferred. Lipofectin or liposomes can be used to  
deliver the antibody or a fragment of the Fab region that binds to the target antigen into  
10 cells. Where fragments of the antibody are used, the smallest inhibitory fragment that  
binds to the target antigen is preferred. For example, peptides having an amino acid  
sequence corresponding to the Fv region of the antibody can be used. Alternatively,  
single chain neutralizing antibodies that bind to intracellular target antigens can also be  
administered. Such single chain antibodies can be administered, for example, by  
15 expressing nucleotide sequences encoding single-chain antibodies within the target cell  
population (see e.g., Marasco *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:7889-7893).

The identified compounds that inhibit target gene expression, synthesis and/or  
activity can be administered to a patient at therapeutically effective doses to prevent, treat  
or ameliorate NARC8 disorders. A therapeutically effective dose refers to that amount of  
20 the compound sufficient to result in amelioration of symptoms of the disorders.

Toxicity and therapeutic efficacy of such compounds can be determined by  
standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for  
determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose  
therapeutically effective in 50% of the population). The dose ratio between toxic and  
25 therapeutic effects is the therapeutic index and it can be expressed as the ratio LD<sub>50</sub>/ED<sub>50</sub>.  
Compounds that exhibit large therapeutic indices are preferred. While compounds that  
exhibit toxic side effects can be used, care should be taken to design a delivery system  
that targets such compounds to the site of affected tissue in order to minimize potential  
damage to uninfected cells and, thereby, reduce side effects.

30 The data obtained from the cell culture assays and animal studies can be used in  
formulating a range of dosage for use in humans. The dosage of such compounds lies

preferably within a range of circulating concentrations that include the ED<sub>50</sub> with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC<sub>50</sub> (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

Another example of determination of effective dose for an individual is the ability to directly assay levels of “free” and “bound” compound in the serum of the test subject. Such assays may utilize antibody mimics and/or “biosensors” that have been created through molecular imprinting techniques. The compound which is able to modulate NARC8 activity is used as a template, or “imprinting molecule”, to spatially organize polymerizable monomers prior to their polymerization with catalytic reagents. The subsequent removal of the imprinted molecule leaves a polymer matrix which contains a repeated “negative image” of the compound and is able to selectively rebind the molecule under biological assay conditions. A detailed review of this technique can be seen in Ansell, R. J. *et al.* (1996) *Current Opinion in Biotechnology* 7:89-94 and in Shea, K.J. (1994) *Trends in Polymer Science* 2:166-173. Such “imprinted” affinity matrixes are amenable to ligand-binding assays, whereby the immobilized monoclonal antibody component is replaced by an appropriately imprinted matrix. An example of the use of such matrixes in this way can be seen in Vlatakis, G. *et al.* (1993) *Nature* 361:645-647. Through the use of isotope-labeling, the “free” concentration of compound which modulates the expression or activity of NARC8 can be readily monitored and used in calculations of IC<sub>50</sub>.

Such “imprinted” affinity matrixes can also be designed to include fluorescent groups whose photon-emitting properties measurably change upon local and selective binding of target compound. These changes can be readily assayed in real time using appropriate fiberoptic devices, in turn allowing the dose in a test subject to be quickly



optimized based on its individual IC<sub>50</sub>. A rudimentary example of such a “biosensor” is discussed in Kriz, D. *et al.* (1995) *Analytical Chemistry* 67:2142-2144.

Another aspect of the invention pertains to methods of modulating NARC8 expression or activity for therapeutic purposes. Accordingly, in an exemplary  
5 embodiment, the modulatory method of the invention involves contacting a cell with a NARC8 or agent that modulates one or more of the activities of NARC8 protein activity associated with the cell. An agent that modulates NARC8 protein activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring target molecule of a NARC8 protein (e.g., a NARC8 substrate or receptor), a NARC8 antibody,  
10 a NARC8 agonist or antagonist, a peptidomimetic of a NARC8 agonist or antagonist, or other small molecule.

In one embodiment, the agent stimulates one or NARC8 activities. Examples of such stimulatory agents include active NARC8 protein and a nucleic acid molecule encoding NARC8. In another embodiment, the agent inhibits one or more NARC8  
15 activities. Examples of such inhibitory agents include antisense NARC8 nucleic acid molecules, anti-NARC8 antibodies, and NARC8 inhibitors. These modulatory methods can be performed *in vitro* (e.g., by culturing the cell with the agent) or, alternatively, *in vivo* (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder  
20 characterized by aberrant or unwanted expression or activity of a NARC8 protein or nucleic acid molecule. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) NARC8 expression or activity. In another embodiment, the method involves administering a NARC8 protein or nucleic  
25 acid molecule as therapy to compensate for reduced, aberrant, or unwanted NARC8 expression or activity.

Stimulation of NARC8 activity is desirable in situations in which NARC8 is abnormally downregulated and/or in which increased NARC8 activity is likely to have a beneficial effect. For example, stimulation of NARC8 activity is desirable in situations  
30 in which a NARC8 is downregulated and/or in which increased NARC8 activity is likely to have a beneficial effect. Likewise, inhibition of NARC8 activity is desirable in

situations in which NARC8 is abnormally upregulated and/or in which decreased NARC8 activity is likely to have a beneficial effect.

The NARC8 molecules can act as novel diagnostic targets and therapeutic agents for controlling one or more of cellular proliferative and/or differentiative disorders, disorders related to aberrant programmed cell death, and neurological disorders.

Additionally, NARC8 molecules may play an important role in the etiology of certain viral diseases, including but not limited to, Hepatitis B, Hepatitis C and Herpes Simplex Virus (HSV). Modulators of NARC8 activity could be used to control viral diseases. The modulators can be used in the treatment and/or diagnosis of viral infected tissue or virus-associated tissue fibrosis, especially liver and liver fibrosis. Also, NARC8 modulators can be used in the treatment and/or diagnosis of virus-associated carcinoma, especially hepatocellular cancer.

#### Pharmacogenomics

The NARC8 molecules of the present invention, as well as agents, or modulators which have a stimulatory or inhibitory effect on NARC8 activity (e.g., NARC8 gene expression) as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) NARC8 associated disorders (e.g., cellular growth related disorders) associated with aberrant or unwanted NARC8 activity. In conjunction with such treatment, pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, a physician or clinician may consider applying knowledge obtained in relevant pharmacogenomics studies in determining whether to administer a NARC8 molecule or NARC8 modulator as well as tailoring the dosage and/or therapeutic regimen of treatment with a NARC8 molecule or NARC8 modulator.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, for example, Eichelbaum, M. *et al.* (1996) *Clin. Exp. Pharmacol. Physiol.* 23(10-

11):983-985 and Linder, M.W. *et al.* (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body (altered drug action) or genetic conditions transmitted as single factors altering the way the body acts on drugs (altered drug metabolism). These pharmacogenetic conditions can occur either as rare genetic defects or as naturally-occurring polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

10           One pharmacogenomics approach to identifying genes that predict drug response, known as "a genome-wide association", relies primarily on a high-resolution map of the human genome consisting of already known gene-related markers (e.g., a "bi-allelic" gene marker map which consists of 60,000-100,000 polymorphic or variable sites on the human genome, each of which has two variants.) Such a high-resolution genetic map can  
15   be compared to a map of the genome of each of a statistically significant number of patients taking part in a Phase II/III drug trial to identify markers associated with a particular observed drug response or side effect. Alternatively, such a high-resolution map can be generated from a combination of some ten million known single nucleotide polymorphisms (SNPs) in the human genome. As used herein, a "SNP" is a common  
20   alteration that occurs in a single nucleotide base in a stretch of DNA. For example, a SNP may occur once per every 1000 bases of DNA. A SNP may be involved in a disease process, however, the vast majority may not be disease-associated. Given a genetic map based on the occurrence of such SNPs, individuals can be grouped into genetic categories depending on a particular pattern of SNPs in their individual genome. In such a manner,  
25   treatment regimens can be tailored to groups of genetically similar individuals, taking into account traits that may be common among such genetically similar individuals.

          Alternatively, a method termed the "candidate gene approach", can be utilized to identify genes that predict drug response. According to this method, if a gene that encodes a drug's target is known (e.g., a NARC8 protein of the present invention), all  
30   common variants of that gene can be fairly easily identified in the population and it can

be determined if having one version of the gene versus another is associated with a particular drug response.

Alternatively, a method termed the "gene expression profiling", can be utilized to identify genes that predict drug response. For example, the gene expression of an animal dosed with a drug (e.g., a NARC8 molecule or NARC8 modulator of the present invention) can give an indication whether gene pathways related to toxicity have been turned on.

Information generated from more than one of the above pharmacogenomics approaches can be used to determine appropriate dosage and treatment regimens for prophylactic or therapeutic treatment of an individual. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a NARC8 molecule or NARC8 modulator, such as a modulator identified by one of the exemplary screening assays described herein.

The present invention further provides methods for identifying new agents, or combinations, that are based on identifying agents that modulate the activity of one or more of the gene products encoded by one or more of the NARC8 genes of the present invention, wherein these products may be associated with resistance of the cells to a therapeutic agent. Specifically, the activity of the proteins encoded by the NARC8 genes of the present invention can be used as a basis for identifying agents for overcoming agent resistance. By blocking the activity of one or more of the resistance proteins, target cells, e.g., cancer cells, will become sensitive to treatment with an agent that the unmodified target cells were resistant to.

Monitoring the influence of agents (e.g., drugs) on the expression or activity of a NARC8 protein can be applied in clinical trials. For example, the effectiveness of an agent determined by a screening assay as described herein to increase NARC8 gene expression, protein levels, or upregulate NARC8 activity, can be monitored in clinical trials of subjects exhibiting decreased NARC8 gene expression, protein levels, or downregulated NARC8 activity. Alternatively, the effectiveness of an agent determined by a screening assay to decrease NARC8 gene expression, protein levels, or downregulate NARC8 activity, can be monitored in clinical trials of subjects exhibiting

increased NARC8 gene expression, protein levels, or upregulated NARC8 activity. In such clinical trials, the expression or activity of a NARC8 gene, and preferably, other genes that have been implicated in, for example, a NARC8-associated disorder can be used as a "read out" or markers of the phenotype of a particular cell.

5

#### Other Embodiments

In another aspect, the invention features, a method of analyzing a plurality of capture probes. The method can be used, e.g., to analyze gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address  
10 of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., a nucleic acid or peptide sequence; contacting the array with a NARC8, preferably purified, nucleic acid, preferably purified, polypeptide, preferably purified, or antibody, and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization  
15 with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the NARC8 nucleic acid, polypeptide, or antibody.

The capture probes can be a set of nucleic acids from a selected sample, e.g., a sample of nucleic acids derived from a control or non-stimulated tissue or cell.

The method can include contacting the NARC8 nucleic acid, polypeptide, or  
20 antibody with a first array having a plurality of capture probes and a second array having a different plurality of capture probes. The results of each hybridization can be compared, e.g., to analyze differences in expression between a first and second sample. The first plurality of capture probes can be from a control sample, e.g., a wild type, normal, or non-diseased, non-stimulated, sample, e.g., a biological fluid, tissue, or cell  
25 sample. The second plurality of capture probes can be from an experimental sample, e.g., a mutant type, at risk, disease-state or disorder-state, or stimulated, sample, e.g., a biological fluid, tissue, or cell sample.

The plurality of capture probes can be a plurality of nucleic acid probes each of which specifically hybridizes, with an allele of NARC8. Such methods can be used to  
30 diagnose a subject, e.g., to evaluate risk for a disease or disorder, to evaluate suitability of a selected treatment for a subject, to evaluate whether a subject has a disease or disorder.

NARC8 is associated with programmed cell death-associated protein activity, thus it is useful for disorders associated with abnormal lipid metabolism.

The method can be used to detect SNPs, as described above.

In another aspect, the invention features, a method of analyzing a plurality of probes. The method is useful, e.g., for analyzing gene expression. The method includes: providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which express or mis express NARC8 or from a cell or subject in which a NARC8 mediated response has been elicited, e.g., by contact of the cell with NARC8 nucleic acid or protein, or administration to the cell or subject NARC8 nucleic acid or protein; contacting the array with one or more inquiry probe, wherein an inquiry probe can be a nucleic acid, polypeptide, or antibody (which is preferably other than NARC8 nucleic acid, polypeptide, or antibody); providing a two dimensional array having a plurality of addresses, each address of the plurality being positionally distinguishable from each other address of the plurality, and each address of the plurality having a unique capture probe, e.g., wherein the capture probes are from a cell or subject which does not express NARC8 (or does not express as highly as in the case of the NARC8 positive plurality of capture probes) or from a cell or subject which in which a NARC8 mediated response has not been elicited (or has been elicited to a lesser extent than in the first sample); contacting the array with one or more inquiry probes (which is preferably other than a NARC8 nucleic acid, polypeptide, or antibody), and thereby evaluating the plurality of capture probes. Binding, e.g., in the case of a nucleic acid, hybridization with a capture probe at an address of the plurality, is detected, e.g., by signal generated from a label attached to the nucleic acid, polypeptide, or antibody.

In another aspect, the invention features, a method of analyzing NARC8, e.g., analyzing structure, function, or relatedness to other nucleic acid or amino acid sequences. The method includes: providing a NARC8 nucleic acid or amino acid sequence; comparing the NARC8 sequence with one or more preferably a plurality of sequences from a collection of sequences, e.g., a nucleic acid or protein sequence database; to thereby analyze NARC8.

Preferred databases include GenBank™. The method can include evaluating the sequence identity between a NARC8 sequence and a database sequence. The method can be performed by accessing the database at a second site, e.g., over the internet.

5 In another aspect, the invention features, a set of oligonucleotides, useful, e.g., for identifying SNP's, or identifying specific alleles of NARC8. The set includes a plurality of oligonucleotides, each of which has a different nucleotide at an interrogation position, e.g., an SNP or the site of a mutation. In a preferred embodiment, the oligonucleotides of the plurality identical in sequence with one another (except for differences in length). The oligonucleotides can be provided with different labels, such that an oligonucleotides  
10 which hybridizes to one allele provides a signal that is distinguishable from an oligonucleotides which hybridizes to a second allele.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are incorporated herein by reference.

15

## EXAMPLES

### Example 1: Identification and Characterization of Human NARC8 cDNAs

The human NARC8A sequence (Figure 1; SEQ ID NO:1), which is  
20 approximately 1507 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 891 nucleotides (nucleotides 368-1261 of SEQ ID NO:1; SEQ ID NO:3). The coding sequence encodes a 297 amino acid protein (SEQ ID NO:2).

The human NARC8A sequence (Figure 2; SEQ ID NO:4), which is  
25 approximately 1380 nucleotides long including untranslated regions, contains a predicted methionine-initiated coding sequence of about 1119 nucleotides (nucleotides 13-1134 of SEQ ID NO:4; SEQ ID NO:6). The coding sequence encodes a 373 amino acid protein (SEQ ID NO:5).

30

## Example 2: Rat NARC8 Induces Programmed Cell Death Induced in Cerebellar Granule Neurons by

Cerebellar granule neurons were transfected with the following enhanced green fluorescent protein (EGFP) expression constructs: Caspase 3-EGFP, Caspase 9-EGFP, EGFP-NARC8 (with the EGFP sequences located at the amino terminus of the fusion protein), and NARC8-EGFP (with the EGFP sequences located at the carboxy terminus of the fusion protein). 48 hours after transfection, the percentage of GFP positive and GFP negative cells undergoing apoptosis was determined by laser-scanning cytometry. The results are given in Table 1. CGN cells transfected caspase 3-EGFP or caspase 9-EGFP showed a 5.3 and 6.1 fold increase in apoptosis, respectively. Both EGFP-NARC8 cells and NARC8-EGFP cells showed an approximate 6 fold increase in apoptosis.

TABLE 1					
Expression Construct	Number of Experiments	Number of GFP + Cells	Mean % Transfection Efficiency	% of GFP- Cells Undergoing Apoptosis	% of GFP+ Cells Undergoing Apoptosis
$\Delta$ EGFP	3	19	0.04%	10.07%	--
Caspase 3-EGFP	3	936	0.78%	7.88%	42.15%
Caspase 9-EGFP	2	378	0.68%	10.08%	61.18%
EGFP-NARC8	3	690	0.51%	8.43%	51.53%
NARC8-EGFP	3	547	0.42%	8.74%	50.20%

## Example 3: Tissue Distribution of NARC8 mRNA

Northern blot hybridizations with various RNA samples can be performed under standard conditions and washed under stringent conditions, i.e., 0.2 X SSC at 65°C. A DNA probe corresponding to all or a portion of the NARC8 cDNA (SEQ ID NO:1) can be used. The DNA was radioactively labeled with  $^{32}\text{P}$ -dCTP using the Prime-It Kit (Stratagene, La Jolla, CA) according to the instructions of the supplier. Filters containing



mRNA from mouse hematopoietic and endocrine tissues, and cancer cell lines (Clontech, Palo Alto, CA) can be probed in ExpressHyb hybridization solution (Clontech) and washed at high stringency according to manufacturer's recommendations.

#### 5 Example 4: Recombinant Expression of NARC8 in Bacterial Cells

In this example, NARC8 is expressed as a recombinant glutathione-S-transferase (GST) fusion polypeptide in *E. coli* and the fusion polypeptide is isolated and characterized. Specifically, NARC8 is fused to GST and this fusion polypeptide is expressed in *E. coli*, e.g., strain PEB199. Expression of the GST-NARC8 fusion protein  
10 in PEB199 is induced with IPTG. The recombinant fusion polypeptide is purified from crude bacterial lysates of the induced PEB199 strain by affinity chromatography on glutathione beads. Using polyacrylamide gel electrophoretic analysis of the polypeptide purified from the bacterial lysates, the molecular weight of the resultant fusion polypeptide is determined.

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#### Example 5: Expression of Recombinant NARC8 Protein in COS Cells

To express the NARC8 gene in COS cells, the pcDNA/Amp vector by Invitrogen Corporation (San Diego, CA) is used. This vector contains an SV40 origin of replication, an ampicillin resistance gene, an *E. coli* replication origin, a CMV promoter followed by  
20 a polylinker region, and an SV40 intron and polyadenylation site. A DNA fragment encoding the entire NARC8 protein and an HA tag (Wilson *et al.* (1984) *Cell* 37:767) or a FLAG tag fused in-frame to its 3' end of the fragment is cloned into the polylinker region of the vector, thereby placing the expression of the recombinant protein under the control of the CMV promoter.

25 To construct the plasmid, the NARC8 DNA sequence is amplified by PCR using two primers. The 5' primer contains the restriction site of interest followed by approximately twenty nucleotides of the NARC8 coding sequence starting from the initiation codon; the 3' end sequence contains complementary sequences to the other restriction site of interest, a translation stop codon, the HA tag or FLAG tag and the last  
30 20 nucleotides of the NARC8 coding sequence. The PCR amplified fragment and the pCDNA/Amp vector are digested with the appropriate restriction enzymes and the vector

is dephosphorylated using the CIAP enzyme (New England Biolabs, Beverly, MA). Preferably the two restriction sites chosen are different so that the NARC8 gene is inserted in the correct orientation. The ligation mixture is transformed into *E. coli* cells (strains HB101, DH5 $\alpha$ , SURE, available from Stratagene Cloning Systems, La Jolla, CA, 5 can be used), the transformed culture is plated on ampicillin media plates, and resistant colonies are selected. Plasmid DNA is isolated from transformants and examined by restriction analysis for the presence of the correct fragment.

COS cells are subsequently transfected with the NARC8-pcDNA/Amp plasmid DNA using the calcium phosphate or calcium chloride co-precipitation methods, DEAE- 10 dextran-mediated transfection, lipofection, or electroporation. Other suitable methods for transfecting host cells can be found in Sambrook, J., Fritsh, E. F., and Maniatis, T. *Molecular Cloning: A Laboratory Manual*. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989. The expression of the NARC8 polypeptide is detected by radiolabelling (<sup>35</sup>S-methionine or <sup>35</sup>S-cysteine 15 available from NEN, Boston, MA, can be used) and immunoprecipitation (Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1988) using an HA specific monoclonal antibody. Briefly, the cells are labeled for 8 hours with <sup>35</sup>S-methionine (or <sup>35</sup>S-cysteine). The culture media are then collected and the cells are lysed using detergents (RIPA buffer, 150 mM NaCl, 20 1% NP-40, 0.1% SDS, 0.5% DOC, 50 mM Tris, pH 7.5). Both the cell lysate and the culture media are precipitated with an HA specific monoclonal antibody. Precipitated polypeptides are then analyzed by SDS-PAGE.

Alternatively, DNA containing the NARC8 coding sequence is cloned directly into the polylinker of the pCDNA/Amp vector using the appropriate restriction sites. The 25 resulting plasmid is transfected into COS cells in the manner described above, and the expression of the NARC8 polypeptide is detected by radiolabelling and immunoprecipitation using a NARC8 specific monoclonal antibody.

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### Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

5 claims.